DISTRIBUTION AND BIOGEOGRAPHY OF THE ALASKAN HARE (*LEPUS OTHUS)*

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# DISTRIBUTION AND BIOGEOGRAPHY OF THE ALASKAN HARE (*LEPUS OTHUS)*

A

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ABSTRACT

The Alaskan Hare (*Lepus othus* Merriam 1900) is the largest lagomorph in North America but remains one of the most poorly studied terrestrial mammals on the continent. Its current distribution is restricted to western Alaska south of the Brooks Range, but historical anecdotal accounts of occurrences north of the Brooks Range (the North Slope) have led to confusion over its past, present, and predicted distribution. To clarify the historical range of *L. othus*, we surveyed North American museum collections and georeferenced voucher specimens (Supplemental File Appendix 1.1). We also located a specimen from the North Slope of Alaska long presumed lost and whose identity had come to be questioned. The rediscovery of this missing specimen suggests the occurrence of at least one Alaskan Hare on the North Slope as recently as the late 1800s.

Because unforested ecosystems such as tundra and Arctic grasslands have decreased in Alaska since the last glacial maximum, and *L. othus* occurs in unforested habitat, we expected to observe low genetic diversity in the mitochondrial control region of *L. othus*. However, with recently collected specimens from regions in Alaska that were poorly represented in the past (i.e. Alaska Peninsula, Little Diomede, and Kotzebue Sound), we discovered more genetic diversity and population structure than was found in previous studies, including similar haplotypes from the Alaska Peninsula and from eastern Russia. This suggests there may have been 2 distinct colonization events of northern hares in Alaska, or introgression from *L. timidus* and a mitochondrial sweep that has been restricted to the Alaska Peninsula and Bristol Bay area. Our morphological analyses of the difference between the two subspecies, *L. o. othus* and *L. o. poadromus*, were ambiguous, with principal components analysis and simple linear regression

indicating the presence of a latitudinal size cline and discriminant function analysis revealing successful group assignment that is not solely based on latitude.

Our research clarifies the current and recent distribution of the Alaskan Hare and reveals more genetic diversity than previously suspected in the mitochondrial control region. We also observed a new biogeographic pattern and closer mtDNA association with *L. timidus*, which, combined with new island specimens and observations, suggests gene flow across the Bering Strait. It also highlights the importance of maximizing sample sizes and sampling widely across a taxon's geographic distribution.

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# DEDICATION PAGE

In dedication to my life partner, Ben McDaniel, for his unwavering belief in me; and to my parents, sister, and grandmother for all their love and support. Finally, all my gratitude to the wonder women scientists who have gone above and beyond the call of duty and friendship when I needed it: Kyndall, Katie E., Katie R., Krystal, and Mallory.

# INTRODUCTION

The Alaskan Hare (*Lepus othus* Merriam 1900) is 1 of only 6 mammal species endemic to the state of Alaska (MacDonald and Cook 2009) and, with an average weight of 4.8 kg (Anderson and Lent 1977), is the largest hare in North America (Feldhammer, Thompson and Chapman 2003). A tundra herbivore, *L. othus* feeds on willow (*Salix* spp.), crowberry (*Empetrum nigrum*), and various tundra evergreens, grasses, sedges, and lichen (Anderson 1974). It is preyed on by both red (*Vulpes vulpes*) and arctic (*V. lagopus*) foxes (Anderson 1974), and golden eagles (*Aquila chrysaetos*; Anderson 1974). *L. othus* remains (University of Alaska Museum [UAM] Mammal Collection specimen 113979) have also been found in gyrfalcon (*Falco rusticolus*) nests. Other likely predators include snowy owl (*Bubo scandiacus*), gray wolf (*Canis lupus*), and rough-legged hawk (*Buteo lagopus*; Anderson 1974). There are currently no harvest restrictions on *L. othus* (Alaska Department of Fish and Game 2014a), which is classified by the State of Alaska as a furbearer and is hunted and trapped for meat and fur.

Potential competition with the only other native leporid in Alaska, the Snowshoe Hare (*Lepus americanus*), has not been studied in detail. The 2 species are sympatric throughout much of western Alaska (MacDonald and Cook 2009) and are frequently confused due to their similar pelage. Both species have white fur in the winter and brown fur in the summer (Best and Henry 1994; Wilson and Ruff 1999). However, Alaskan Hares can be easily identified in their winter pelage by fur that is white to the roots while winter pelage of *L. americanus* is white tipped with dark roots. The 2 species also differ significantly in size. Snowshoe Hares weigh 0.9-2.2 kg and have a total length 363-520 mm (Wilson and Ruff 1999). Alaskan Hares weigh 3.9-6.5 kg and have a total length 570-690 mm (Anderson 1974). *L. americanus* occurs throughout most of mainland Alaska except for the western half of the Seward Peninsula and all but the easternmost

end of the Alaska Peninsula (MacDonald and Cook 2009), while *L. othus* is restricted to coastal western Alaskan (MacDonald and Cook 2009).

*Distribution.— Lepus othus* is restricted to the coast of western Alaska, a sparsely populated, unforested region far removed from the state's road network. Alaska's other expanse of tundra lies north of the Brooks Range (the North Slope hereafter), which is the northernmost mountain range and extent of treeline in the United States (Walker et al. 2005). *L. othus* is not thought to presently occur north of the Brooks Range, but some authorities (Bee and Hall 1956; Hall 1981) have included the North Slope in published range maps.

The 1898 Arctic expedition led by Edward Avery McIlhenny (son of Edmund McIlhenny, inventor of Tobasco™ brand pepper sauce) acquired a large hare specimen from near Point Barrow from well-known trader Charles Brower (Stone 1900; Howell 1936). The fate of this specimen has long been unknown and subsequent researchers have been unable to confirm its identification (Anderson 1978). At the time, the skeleton was identified as *L. tschuktschorum* Nordquist 1883 (Stone 1900), a junior synonym of *L. timidus* that included hares in Eastern Siberia and Alaska. Reported measurements (Stone 1900) indicated the skeleton was too large to be *L. americanus*, and Snowshoe Hares were not observed in the area until several decades later (MacDonald and Cook 2009). Out of 317 known or purported Alaskan Hare specimens ever collected, it remains the only purported voucher from north of the Brooks Range. Aside from this single specimen, Brower wrote in a letter that he had "never known [Alaskan Hares] to live on the low tundra anywhere near the [Arctic] coast" (Howell 1936:334). From the skins brought to him by Alaska Natives, Brower presumed (Howell 1936) *L. othus* to be more common inland on the North Slope and knew of Alaskan Hares near Cape Lisburne and Point Lay, coastal villages immediately north of the western terminus of the Brooks Range.

North Slope sightings reported by Bee and Hall (1956) date from 1882 and continued intermittently until 1950 when a United States Geological Survey crew saw "two rabbits [that] were large and probably were [Alaskan] hares" at the head of the Colville River (Bee and Hall 1956:32). Hall (1981) later inexplicably recognized only 2 out of 14 of these anecdotal localities despite the lack of any new *L. othus* sightings or specimens from the North Slope.

Alaskan Hares do not appear to have been common inland from the Kotzebue area for the past century and are "exceedingly rare and seldom taken" in the region (Nelson 1998:295). Rausch (1951:178) raised the possibility of *L. othus* occurring "near the northern limit of the Brooks Range." Nunamiut Eskimos reported having seen *L. othus* near Umiat (Rausch 1951), which is east of most other reported sightings. There are no specimens from the Umiat area and Rausch reported no sightings of his own, despite his frequent visits. Accounts of *L. othus* from Point Lay to Point Barrow extend north of the Brooks Range in the late 1800s and early 1900s (Anderson 1978), but there are no indications *L. othus* currently occurs there.

*Taxonomic history.— Lepus othus* is part of a species complex comprising the subgenus *Lepus* Linnaeus 1758, which currently includes 2 other northern hares: the Arctic Hare (*L. arcticus* Ross 1819) of Canada and Greenland, and the Mountain Hare (*L. timidus* Linnaeus 1758) of northern Eurasia. Currently, geography is used to differentiate the 3 species; morphological (Baker et al. 1983; Dixon et al. 1983) and molecular (Waltari and Cook 2005) data have been inconclusive or conflicting, and the taxonomy of northern hares remains unclear.

*Lepus othus* Merriam 1900 was originally given the common name tundra polar hare and has 1 recognized junior synonym, the peninsula arctic hare (*Lepus poadromus* Merriam 1900), which was subsequently subsumed into *L. othus* by Howell (Howell 1936). The skull of *L. othus* was described as larger than that of *L. arcticus* from Canada and eastern Arctic America, but

comparable to *L. timidus* from eastern Russia, and with darker summer pelage on the head than that of *L. timidus* from eastern Russia (Merriam 1900).

In his revision of North American hares, Howell (1936) examined 48 specimens of *L. o. othus*; 14 of *L. o. poadromus*; over 300 of *L. arcticus* from across northern Canada and Greenland, but only 2 skulls and 1 skin in summer pelage of *L. timidus* from Siberia. He recognized *L. o. othus* and *L. o. poadromus* as valid subspecies, and *L. othus* distinct from *L. arcticus* and "closely related" to but distinct from *L. timidus* (Howell 1936:333). *L. o. tschuktschorum* was later accepted (Hoffmann and Smith 2005) as the name of the southern *L. othus* subspecies instead of the more commonly used *L. o. poadromus*. It has been argued that if *L. timidus* individuals in eastern Siberia are more genetically similar to *L. othus* than to other *L. timidus* populations farther west, the species name *L. tschuktschorum* would have priority (Hoffmann and Smith 2005).

In his detailed review of the systematics of the Alaskan Hare, Anderson (1974) recognized *L. othus*, *L. arcticus*, and *L. timidus* as distinct species. He recorded 7 skull measurements from 144 *L. othus* specimens, 25 *L. arcticus* specimens, 34 *L. timidus* specimens, and 10 *L. townsendii* specimens (as an outgroup). He determined Mahalanobis' generalized distances between *L. othus* and the other 2 northern hare species were greater than the minimum generalized distance between the northern hares and outgroup *L. townsendii*. Multiple discriminant analysis correctly classified 92% of the specimens. Anderson determined the means of all 7 skull and 6 body measurements of *L. othus* to be larger than those of *L. arcticus* and *L. timidus*, which is evidence against a size cline across the 3 species (*L. timidus* occurs at similar latitudes as *L. othus* while *L. arcticus* occurs at higher latitudes).

Principal components analysis (PCA) of 20 craniodental measurements from 831 skulls representing all 3 nominal taxa led Baker et al. (1983) to recognize all populations from Greenland, Canada, Alaska, and Russia's Chukotsk Peninsula as *L. arcticus* and those from the rest of Eurasia as *L. timidus*. Their multivariate analyses suggested that the single specimen from the Chukotsk Peninsula was phenetically more similar to hares in Alaska as well as those from Banks and Prince Patric Islands, Canada. Based on PCA and cluster analysis of 12 craniodental measurements, Dixon et al. (1983) concluded that the 3 northern hare taxa were conspecific and likely represented a circumpolar ring species (*L. timidus* [Flux 1983]). Ramos (1999) measured 18 maxillary and mandibular characters in 85 *L. arcticus*, 102 *L. othus*, and 44 *L. timidus* skulls and performed a discriminant analysis that recovered all 3 northern hare species as distinct clusters, although she did not draw any taxonomic conclusions.

Waltari et al. (2004) recognized *L. othus*, *L. arcticus*, and *L. timidus* as distinct based on DNA sequences from the mitochondrial control region of 95 northern hare specimens. Waltari and Cook (2005) later added mitochondrial control region sequences from 97 additional specimens and maintained support for the species status of *L. othus* (Waltari and Cook 2005). Melo-Ferreira et al. (2012) sequenced 14 nuclear and 2 mitochondrial loci from 6 *L.* timidus, 3 *L. arcticus*, and 2 *L. othus* specimens, and their results did not refute the recognition of northern hares as 3 distinct species. Although northern hare taxonomy remains uncertain, for the time being we recognize *L. othus*, *L. arcticus*, and *L. timidus*.

*Natural history.*—Much of the published information on the behavior and ecology of *L. othus* is inferred from the better-studied *L. arcticus* and/or *L. timidus* (Anderson 1974). Little is known about the Alaskan Hare's dispersal behavior or home range size, factors that likely influence its distribution and phylogeography. Dispersal of *L. arcticus* and *L. timidus* have been

shown to vary widely depending on geography, sex, season, and food availability (Hewson and Hinge 1990; Kauhala, Helle and Hiltunen 2005). Tracks at Peel Point, northwest Victoria Island, Canada, indicated groups of Arctic Hares crossed at least 20 km of sea ice from Banks Island (Manning and Macpherson 1958). *L. arcticus* has been observed on sea ice as far as 32 km offshore (Howell 1936) and is estimated to be capable of surviving solely off fat stores at -24°C for up to 15 days (Wang et al. 1973). Their extremely effective insulation likely aids in long-distance dispersal across a landscape with uncertain browse availability. Given their nearly identical morphology and recent (and perhaps incomplete) divergence, it seems likely that *L. othus* and *L. timidus* are similarly adapted. Dispersal distances vary widely among *L. timidus* individuals but the maximum dispersal distance measured was 200 km after a captive hare was release in Scandinavia (Angerbjörn and Flux 1995). However, unlike *L. othus* and *L. arcticus*, *L. timidus* occurs in temperate and boreal habitats in addition to tundra (Hamill, Doyle and Duke 2006; Newey et al. 2007). Home range size and dispersal habits of *L. timidus*, therefore, may not be analogous to those of the other 2 species.

*Lepus timidus* occurs in forested habitat across much of temperate Eurasia (Thulin 2003), where *L. americanus* is absent and its primary leporid competitor is the European hare, *L. europaeus.* The European hare thrives in cleared, open agricultural land, restricting *L. timidus* to forest and tundra where the 2 occur in parapatry. The southern range limit of mountain hares in Scandinavia has shifted north since the introduction of European hares in the late 19$^{th}$ century (Thulin 2003). This may represent another example of a northern hare being replaced by a southern congener.

*Lepus othus* was designated a Species of Greatest Conservation Concern by Alaska's Comprehensive Wildlife Conservation Strategy (Alaska Department of Fish and Game 2006),

due largely to the uncertainty surrounding its distribution and taxonomic status (see below). The International Union for Conservation of Nature (IUCN) currently classifies *L. othus* as Least Concern because "populations seem to be healthy" (Murray and Smith 2008). However, there is no published research on population status or trends in the species. In 1995 there were accounts (Klein 1995:359) that "populations from Kotzebue to the Yukon-Kuskokwim Delta have remained low since population highs in the 1970s, whereas hare densities on the Alaska Peninsula have been reported low since the early 1950s", but there have been no quantitative studies.

We conducted an extensive review of the present distribution of *L. othus* based on museum specimens, photographs, literature records, and credible observations. We also evaluated the population structure and mitochondrial diversity within *L. othus* and expanded Anderson's (1974) craniometrics dataset to test subspecies boundaries with 97 additional specimens collected since his research was conducted.

CHAPTER 1:

# Revised distribution of an Alaskan endemic, the Alaskan Hare (*Lepus othus*), with implications for taxonomy, biogeography, and climate change[1]


## ABSTRACT

The Alaskan Hare (*Lepus othus* Merriam 1900) is the largest lagomorph in North America but remains one of the most poorly studied terrestrial mammals on the continent. Its current distribution is restricted to western Alaska south of the Brooks Range, but historical accounts from north of the Brooks Range (the North Slope) have led to confusion over its past, present, and predicted future distributions. To determine if *L. othus* occurs or historically occurred on the North Slope, we surveyed museum collections, vetted observational accounts, and produced a spatial distribution model based on the resulting georeferenced records. We located a historic specimen long presumed lost that suggests the occurrence of *L. othus* on the North Slope as recently as the late 1800s. We also uncovered evidence of *L. othus* and/or Mountain Hares (*L. timidus*) on several islands in the Bering Sea, raising the possibility of recurring gene flow between these closely related species across seasonal ice connecting Asia and North America. While our results paint a more complete picture of the current distribution of *L. othus*, persistent uncertainties surrounding its taxonomic status and potential northward range shift onto lands reserved for oil and gas development call for additional study.


[1] Cason, M. M., A. P. Baltensperger, T. L. Booms, J. J. Burns, and L. E. Olson. In review. Revised distribution of an Alaskan endemic, the Alaska Hare (*Lepus othus*), with implications for taxonomy, biogeography, and climate change. Arctic Science.

# INTRODUCTION

The Alaskan Hare (*Lepus othus* Merriam 1900) is the largest hare in North America (eds. Feldhammer et al. 2003) and is the only terrestrial vertebrate with a distribution restricted to the vast, roadless, unforested region of western Alaska south of the Brooks Range (MacDonald and Cook 2009). Despite these distinctions, little is known about its natural history, ecology, population dynamics, or distribution. Far more research has been conducted on the two other species in the northern complex, the Arctic Hare (*L. arcticus* Ross 1819) from northern Canada and the Mountain Hare (*L. timidus* Linnaeus 1758) from northern Eurasia, each of which occupies a much larger current range than does *L. othus*. For the purpose of disambiguation, we follow MacDonald and Cook [2009] in referring to *L. othus* as the "Alaska" and not "Alaskan" Hare, since there are two hare species native to Alaska and therefore two "Alaskan hares." Similarly, we capitalize formal common names.

The unique distribution of *L. othus* has been the source of confusion for over a century (Fig. 1.1). Until now, there has been no verifiable evidence of the occurrence of *L. othus* on the tundra north of the Brooks Range (the North Slope hereafter), but historical anecdotal accounts dating from the late 19th through the mid-20th century (Bee and Hall 1956) continue to influence published range maps (e.g., Feldhammer et al. 2003; Klein 1995; Wilson and Ruff 1999; Fig. 1.1). The only specimen purportedly collected from the North Slope was acquired by the 1898 Arctic expedition led by Edward Avery McIlhenny, who obtained a large hare from near Point Barrow (Stone 1900). The fate of this specimen has long been unknown and subsequent researchers have been unable to confirm its identification (Anderson 1978) and have even reported it lost (Bee and Hall 1956). As a result, no North Slope records or specimens were included in MacDonald and Cook's (2009) distribution map of *L. othus*. Reported measurements

(Stone 1900) indicated the skeleton was too large to be *Lepus americanus*, leading Anderson (1978) to provisionally identify the specimen sight unseen as *L. othus*. Out of 318 *L. othus* specimens available for study, it remains the only purported voucher from anywhere north of the Brooks Range.

The most comprehensive study of the natural history and systematics of *L. othus* (Anderson 1974) included a map of all known the collection localities of the 189 then-known specimens. The range map encompassed the west coast of Alaska, from the southern Alaska Peninsula to the Kotzebue Sound region, but did not include the North Slope (Anderson 1978). By 1974 there had been no additional sightings of *L. othus* on the North Slope, "despite increased biological investigations associated with recent oil and gas activities" (Anderson 1978, p. 73). Industrial development on the North Slope has increased since the 1970s, along with the biological research required to assess environmental impacts. To date there have been no sightings reported from the North Slope since 1950 and no large-bodied hare specimens collected since 1898.

Because no voucher specimens could be located, the historical occurrence of *L. othus* north of the Brooks Range has been uncertain. Forest cover is expected to increase and low shrub habitat is expected to decrease in northwest Alaska over the next century (Jorgenson et al. 2015). *Lepus othus* is projected to lose 5% of its habitat in the national public lands of the region (Bering Land Bridge National Preserve, Cape Krusenstern National Monument, Gates of the Arctic National Park and Preserve, Kobuk Valley National Park, Noatak National Preserve, and Selawik National Wildlife Refuge) by the year 2100 (Marcot et al. 2015). However, other species distribution models (Leach et al. 2015) predict an 80% increase in the bioclimatic

envelope of *L. othus* between the beginning of the 20th and end of the 21st centuries, along with a 3% mean latitudinal increase.

*Lepus othus* feeds on willow (*Salix* spp.), crowberry (*Empetrum nigrum*), and various other ericaceous plants, grasses, sedges, and lichens (Anderson 1974). It is preyed on by both Red Fox (*Vulpes vulpes*) and Arctic Fox (*V. lagopus*), and Golden Eagles (*Aquila chrysaetos*; Anderson 1974). *Lepus othus* remains (University of Alaska Museum [UAM] Mammal Collection specimen 113979) have also been found in Gyrfalcon (*Falco rusticolus*) nests. Other likely predators include Snowy Owl (*Bubo scandiacus*), Gray Wolf (*Canis lupus*), and Rough-legged Hawk (*Buteo lagopus*; Anderson 1974).

Potential ecological interactions between *L. othus* and the only other native leporid in Alaska, the Snowshoe Hare (*Lepus americanus* Erxleben 1777), has been suggested but not directly studied (Klein 1995). The two species are sympatric throughout much of western Alaska (MacDonald and Cook 2009; Fig. 1.2) and are frequently confused due to their similar pelage. *Lepus americanus* occurs throughout most of mainland Alaska except the western half of the Seward Peninsula and all but the easternmost terminus of the Alaska Peninsula (Alaska Department of Fish and Game; MacDonald and Cook 2009) while *L. othus* is restricted to coastal western Alaskan (MacDonald and Cook 2009).

In light of the inordinately rapid pace of environmental change in Alaska's arctic and coastal tundra regions, the unique and restricted yet poorly understood range of *L. othus*, and the four decades that have elapsed since the distribution of this Alaskan endemic was last rigorously evaluated, we conducted an extensive review of the present distribution of *L. othus*. We hypothesized that there is no evidence to include the North Slope in the present distribution of *L. othus*, and that the historical anecdotal accounts from the North Slope could not be corroborated

by voucher specimens. Our review was based on museum specimens, photographs, literature records, and credible observations. We also compared the known distribution based on these data to a spatial distribution model for *L. othus*.

## MATERIALS AND METHODS

We surveyed 39 North American and Canadian museums (Table 1.1) for *L. othus* specimens through online database searches, contact with curators and collection managers, and/or personal visits. All known *L. othus* specimens are listed in Supplemental File Appendix 1.1. Museums surveyed but not listed in the Supplemental File Appendix 1.1 had no *L. othus* specimens. We confirmed identifications by skull size and/or winter pelage coloration, through personal inspection or photos from curators and collection managers. Where neither was possible, we considered the familiarity of the collector or identifier with both *L. othus* and the only other hare known to occur in sympatry and with which it is occasionally confused, *L. americanus*. *Lepus othus* in its winter pelage is easily identified by distinctive black-tipped ears and fur that is white to the roots. Winter pelage of *L. americanus* is white tipped with dark roots and does not include black fur on the ventral tips of the pinnae (although black may be present along the edges).

In addition to traditional voucher specimen, we included accounts of *L. othus* catalogued and curated as observations in the Mammal Collection of the University of Alaska Museum (UAM). We evaluated these records on a case-by-case basis prior to acceptance as catalogued records and must generally include photographs or video to corroborate identification. In some cases observer expertise alone was considered sufficient following interviews by museum staff. Catalog numbers for UAM observations are denoted with the prefix "UAMObs".

*Lepus othus* specimens with collection locality descriptions but without geographic coordinates were assigned coordinates (WGS 84 datum) and error radii with the georeferencing platform GEOLocate (Rios and Bart 2014). Misspelled place names were corrected according to the Dictionary of Alaska Place Names (Orth 1971). Locality descriptions that were not recognized by GEOLocate were manually assigned coordinates and error radii with Google Earth Pro v. 7.0.3.8542. Error radii were determined according to BioGeoMancer standards for estimating error from locality descriptions (Chapman and Wieczorek 2006). Locations with error radii exceeding 200 km were georeferenced but not included in Figure 1.3. Place names or descriptions that could not be located were not assigned coordinates and are not shown in Fig. 1.3, but are included in the Supplemental File 1.1. We georeferenced all specimens collected between 1877 (the earliest known modern *L. othus* specimen) and 2014. We did not examine paleontological or zooarchaeological material, which tends to be fragmentary and identified in the literature and/or on collections databases to genus level only.

We used RandomForests (Salford Systems, Inc., San Diego, CA, USA; www.salford-systems.com) to create a spatial distribution model for *L. othus*. The resulting model accounts for the confounding and non-linear relationships among variables (Breiman 1996, Cutler et al. 2007). Collection localities with accuracy within 100 km were used as presence points for the spatial distribution model. Presence locations and 500 randomly-distributed pseudo-absence points for *L. othus* were attributed with 28 environmental predictor layers (Table 1.2) using the extract values to multi-point tool in ArcGIS 10.3.1 (ESRI, Inc., Redlands, CA, USA), and used as training data to develop the model in RandomForests. The model was grown to 1000 trees, considered eight predictors at each node, and used all other software default settings. Aspatial performance was cross-validated internally in RandomForest using an 'out-of-bag' set of training

points (Breiman 1996). The discrimination capacity of each model was assessed using resultant sensitivity and specificity of the out-of-the bag dataset and the area under the curve (AUC) based on the receiver-operating characteristic (ROC). The model was applied in ArcGIS 10.3.1 to a grid of points distributed at 1-km intervals across Alaska that were also attributed with the same environmental predictors. Model outputs generated relative indices of occurrence (RIO) for each point, which is a ranking of pixels from 0 to 1 representing the likelihood of belonging to the 'presence' class. A balanced threshold of 0.55 was used to differentiate between presences and absences for all models. For continuous visualization between points, predicted RIO values were rastered using the Inverse Distance Weighting (IDW) tool using a 1-km resolution and clipped to the state coastline. RandomForests was also used to rank the relative importance of the environmental variables in the model.

## RESULTS

We located 318 specimens of *L. othus* from 12 museum and other collections and were able to georeference 305 from their locality descriptions (Fig. 1.3). Many were already associated with geographic coordinates available from the host museum. These localities spanned much of Alaska's west coast but the majority (175) of specimens were collected from the Seward Peninsula (Fig. 1.3). The current northernmost range limit was extended approximately 50 km north after collection of the first *L. othus* voucher (UAM 120460) from Noatak National Preserve in April 2014. There was also a credible sighting (UAMObs 213) of an *L. othus* individual in 1978 in the western Brooks Range approximately 150 km northeast of where UAM 120460 was collected. The southernmost specimens were collected in Cold Bay, approximately 50 km from the terminus of the Alaska Peninsula, as recently as 1993 (UAM 42143). We personally confirmed the species identification of the northern- and southernmost

specimens, both of which are housed at UAM. Most specimens were collected close to Alaska's western coastline. The inland-most specimens were collected near St. Mary's, less than 150 km from the coast. Several reliable observations (UAMObs 219-225) originated near Iliamna Lake, which is farther east than almost all specimen collection localities but less than 100 km inland from the Gulf of Alaska.

We located the 'lost' McIlhenny specimen (CMN 31930), a skull and postcranial skeleton collected from near Barrow, in the Mammal Collection at the Canadian Museum of Nature. Data on the specimen tag confirm it is the same specimen listed in the expedition's report (Stone 1900). We borrowed the specimen and verified the craniodental measurements were within the range of the *L. othus* specimens measured by Anderson (1974) and exceeded the maximum basilar skull length of *L. americanus* (67 mm) reported by Hall (1981). Visual comparisons also confirmed that it is not *L. americanus*.

Two new credible observations of *L. othus* on Unimak Island (the easternmost of the Aleutian Islands) in 2013 and 2014 (UAMObs 182, UAMObs 192) were reported, and a photograph of an *L. othus* individual on Hagemeister Island (UAMObs 181) was taken by a camera trap at a walrus haulout site in 2013. Both Hagemeister and Unimak are close to mainland Alaska (<5 km and <1 km, respectively) and are sometimes connected to the mainland by sea ice in late winter and early spring. Additionally, one or more very large hares fitting the description of either *L. timidus* or *L. othus* were reported from St. Lawrence Island (Fig. 1.3) in the winter of 2012 (G. Sheffield, pers. comm.), and are the first known sighting of a hare on that island. Prior to this study, Alaskan Hares were not known from any of these islands.

Russia's Chukotsk Peninsula and Alaska's Seward Peninsula are 80 km apart. Midway between them lie the two Diomede Islands. Large-bodied hares currently identified as *L. othus*

have been collected from Little Diomede Island in 1936 (USNM 260900) and more recently in 2014 (UAM 120797, UAM 122839) but have only been sporadically reported from the island. During winter 2013-2014, several hares were observed on Little Diomede (including the two UAM specimens). UAM 120797 was pregnant with 7 near-term embryos when shot by subsistence hunters in June 2014. Little Diomede lies 37 km west of Alaska's Seward Peninsula and less than 4 km east of Russia's Big Diomede Island, which in turn is only 35 km east of the Chukotsk Peninsula; all are interconnected by sea ice for up to 6 months out of the year (NSIDC 2013). In the 1960s, bush plane pilot Bill Munz reportedly saw a "huge" aggregation of large-bodied hares moving east on the sea ice between Little Diomede Island and Cape Prince of Wales (J. Jacobson, pers. comm.; Fig. 1.3), which is on the western tip of the Seward Peninsula. Whether the hares collected from Little Diomede in 2014 represent *L. othus* from Alaska or *L. timidus* from Russia remains unclear, as does the present occurrence of hares on Big Diomede. However, residents on Little Diomede recounted the regular occurrence of hares on Big Diomede and sporadic dispersal to Little Diomede (J. J. Burns, pers. comm.) in the 1960s.

Curiously, no specimens or observations of hares are known from Nunivak Island (Fig. 1.3), the second-largest island in the Bering Sea and only 30 km from mainland western Alaska, to which it is seasonally connected by sea ice. Residents and hunting guides on Nunivak report that hares do not occur on the island (W. Don, pers. comm.). Our spatial distribution model (Fig. 1.4) predicts their likely presence on Nunivak, but the reason(s) for the apparent absence of *L. othus* there remains unknown.

Olaus Murie noted the absence of *L. othus* on Unimak Island (Fig. 1.3) after his faunal survey of the Alaska Peninsula and Aleutian Islands in the 1930s (Murie 1959). MacDonald and Cook (2009) report no island records from southwest Alaska, aside from a failed introduction on

Chirikof Island (Fig. 1.3). However, one specimen (USNM 203278; identification confirmed by LEO) is listed as having been collected from Sand Point (Fig. 1.3), on Popov Island, in 1913. Popov Island is not surrounded by sea ice in the winter, and ocean currents in the Unga Strait separating the island from the southern coast of the Alaska Peninsula are strong and constant. The village of Sand Point was, like Barrow, a trading center for many years; it's possible the specimen may have been collected on the mainland and brought to Sand Point. Alaskan Hares are not known to occur on Popov Island today, and Snowshoe Hares introduced to the island in 1955 are reported to be abundant (MacDonald and Cook 2009; D. Watts, pers. comm.). The two observations of *L. othus* on Unimak (UAMObs 182, UAMObs 192) and a photograph of *L. othus* on Hagemeister (UAMObs 181) are additional evidence of *L. othus* on islands in southwestern Alaska.

The spatial distribution model closely matched the known distribution based on specimen and observation localities (Fig. 1.4). The ROC value was 0.98, the balanced error rate was 0.06, the sensitivity was 94.24%, and the specificity was 94.00%. Distance to March sea ice and the distance to the coastline were the most important variables in the model (Table 1.2). The northern-most specimen collection locality is in an area of likely *L. othus* presence, as predicted by the spatial distribution model. However, the location of the northern-most credible observation is in the midst of the Brooks Range, where the model predicts the absence of *L. othus*. The model did predict the presence of *L. othus* on St. Lawrence Island, despite the pseudo-absence localities used in the dataset. We did not include the observation of a large-bodied hare on St. Lawrence Island as a presence point because we received it third-hand and it is not considered a catalogued observation.

## DISCUSSION

Based on the collection locality of UAM 120460, we consider the current northernmost known range limit of *L. othus* to be approximately 50 km northeast of Kotzebue. Prior to the acquisition of this specimen and the 'rediscovery' of CMN 31930 from the North Slope, the northernmost *L. othus* voucher was collected in the Kotzebue area (UAM 4132). In May 1978 a *L. othus* individual was sighted 190 km northeast of Kotzebue, well into the Brooks Range (UAMObs 213), by a professional hunting guide very familiar with both *L. othus* and *L. americanus*. The spatial distribution model expects the northernmost range extent of *L. othus* to be near Kivalina, which is about 105 km northwest of the northernmost collection locality. We consider the range of *L. othus* to include the area in which the species is consistently found while acknowledging what are likely dynamic boundaries, particularly along the northern limit of its core range. Therefore, we do not consider the current distribution of *L. othus* to include the North Slope. The absence of *L. othus* on the vast tundra landscape of Alaska's North Slope remains vexingly inexplicable and warrants thorough exploration.

The McIlhenny expedition reported the "Ikpikpun river" as the collection location of the North Slope specimen (Stone 1900, p. 30). However, the donor of the specimen, Charles Brower, reported having the hare brought to him in 1897 from "inland on the Chipp River" and later giving "the whole animal to E. A. McIlhenny, who sent it out with his collection" (Howell 1936, p. 334). Although we could not locate an "Ikpikpun River" on contemporary maps of Alaska, the Ikpikpuk River is about 50 km east of the Chipp River and lies approximately 100 km SE of Point Barrow. The Smithsonian Contributions to Anthropology described the location of the Ikpikpun River as east of Point Barrow (Stanford 1976) and Murdoch (1892) described it as about 40 miles east of the Meade River, which is the approximate location of the Ikpikpuk River.

We therefore conclude that the specimen reported by both Brower and the expedition (stationed in Point Barrow between August 1897-1898) are one and the same.

Long-distance dispersal (200 km) has been documented in *L. timidus* (Angerbjörn and Flux 1995) and it is likely *L. othus* has occasionally dispersed well outside of the species' current core range. According to both the expedition report (Stone 1900) and Brower (Howell 1936, p. 334), CMN 31930 was collected on the North Slope and not transported there by hunters or traders (although not an Alaska Native, Brower spoke fluent Iñupiaq). It is possible Brower's understanding of the collection locality was incorrect and the hare was killed elsewhere and brought to the North Slope, perhaps via a series of trades. However, the fact that external measurements were recorded (Stone 1900) indicate the specimen was an intact carcass available to either the expedition or to Brower. An entire *L. othus* brought to the Point Barrow region from the closest point of its current distribution (about 500 km away) seems cumbersome but possible, especially with winter sled dog travel when the hare could be kept frozen and long-distance travel was common (Burch 1988). If, however, it was collected at its reported location, it may represent an anomalous disperser from northwest Alaska that made its way to the North Slope along the coast.

In their seminal but contentious book, Bee and Hall (1956) included the North Slope in the range of *L. othus* (Fig. 1.1). However, they did not examine any specimens to vouch for a North Slope occurrence, and others (Buckley and Scott 1957) criticized the authors' limited field research and dependence on personal communication. The McIlhenny specimen from near Point Barrow (which Bee and Hall proclaimed lost or misplaced) was the only evidence to corroborate any of Bee and Hall's North Slope records, the other 13 localities represented second- or third-hand accounts. Our exhaustive search did not locate any specimens to confirm Bee and Hall's

other locality records. The McIlhenny specimen provides the only verifiable evidence that at least one Alaskan Hare occurred on Alaska's North Slope in the late 19th century.

Questionable field identifications may have contributed to anecdotal reports of *L. othus* on the North Slope. John Murdoch, an early naturalist and source for Bee and Hall (1956), reported that Alaska Natives in Point Barrow were unfamiliar with *L. othus* but that the "Nunatangmeau" Eskimos brought hare skins there for trade. He considered these skins evidence of "polar hares" occurring "somewhere in the Colville Region" (Murdoch 1885:103). However, the dark roots of the fur on these skins, which are catalogued (USNM E89915-0, USNM E89915-1) in the Anthropology Collection of the United States National Museum of Natural History, confirm that they are *L. americanus*. It is likely the authors of many early reports may not have been able to differentiate between *L. othus* and *L. americanus*, or between traveling Alaska Native groups, which casts further doubt on the validity of these records.

Another possible *L. othus* specimen from the North Slope resides at the California Academy of Sciences (CAS 23818). The specimen is a discolored pelt without head or feet but with pelage resembling that of *L. othus*. According to the limited associated data, it was purchased from a trader in Barrow, Alaska in the early 1960s. The skin tag identifies the specimen as an "Arctic Hare *Lepus arcticus*," a close relative of *L. othus* that ranges across much of northern Canada does not occur in Alaska. However, the term "arctic hare" to this day is commonly, if erroneously, used to refer to *L. othus*. If the animal was collected in the Barrow area, it was likely *L. othus*. The hide was tanned, a process that significantly damages DNA, and we cannot conclusively identify the specimen as *L. americanus*, *L. othus*, or *L. arcticus* on visual inspection. In light of the uncertain provenance and identification we do not consider it to be a credible voucher.

Zooarchaeological evidence of *L. othus* has been reported from Cape Thompson (Fig 1.3; Pruitt Jr. 1966), where bones and teeth identified as *L. othus* were discovered in the remains of 150- to 200-year-old Eskimo dwellings. However, it is unclear if *L. othus* actually occurred at Cape Thompson or was hunted elsewhere and brought there. Other archaeological and zooarchaeological remains from Alaska include numerous specimens identified as "*Lepus* sp." or as "*Lepus othus* or *Lepus americanus*" (AMNH:FAM:99926; Yesner 2001). Identification of these remains would provide valuable insight into the past distribution of both species.

No other extant mammal is restricted to coastal western and southwestern Alaska without also being found on the North Slope (MacDonald and Cook 2009), and it is unclear why the current distribution of *L. othus* is apparently limited to south of the Brooks Range. The closely related and morphologically indistinguishable *L. arcticus* is found much farther north in Canada (Fig. 1.2) in more extreme environments than are found on Alaska's North Slope. The presumed expansion of *L. americanus* to the North Slope indicates that the mountains of the Brooks Range are not a significant barrier to hares. The 1978 sighting of *L. othus* well within the western Brooks Range (UAMObs 213) may indicate the occurrence of occasional long-distance dispersers north of the current range extent or step-wise dispersal over multiple generations. Although there is no apparent biogeographic barrier, the western distribution of *L. arcticus* likewise appears to end abruptly in the Northwest Territories, Canada, and does not extend to Alaska's North Slope. Waltari et al. (2004) concluded that the Mackenzie River, in northwest Canada, is a boundary between the two species, which may have expanded from two different glacial refugia, as has been hypothesized for small mammals such as the Collared Lemming, *Dicrostonyx groenlandicus* (Fedorov and Stenseth 2002). Porsild (1945) reported unspecified evidence he attributed to *L. arcticus* in the Richardson Mountains west of the MacKenzie River

in July 1933 and on gravel ridges in the foothills between the Mackenzie Delta and the Alaska Border. However, it remains unclear why northern hares, medium-sized mammals that have been recorded dispersing over great distances (Angerbjörn and Flux 1995), do not occur on the tundra north of the Brooks Range. The spatial distribution model does not predict the presence of *L. othus* on the North Slope, which may be unfavorable to northern hares for as-yet unknown reasons, such as snow pack and terrain.

It is unclear if the apparent northward expansion of *L. americanus* has affected the geographic range of *L. othus*. There is little mention of *L. americanus* north of the Brooks Range before the 1990s (Klein 1995, MacDonald and Cook 2009) except along waterways with willows "at times of high population" (Manville and Young 1965, p. 16). Tape et al. (2015) calculated that shrub height in northern Alaska reached the threshold required by *L. americanus* between 1964 and 1989.

Unlike *L. arcticus* and *L. othus*, *L. timidus* is found in boreal forest in addition to alpine and tundra habitat, possibly because *L. americanus*, which thrives in the boreal forests of North America, does not occur in Eurasia. *Lepus arcticus* can survive at low densities in shrub or partially forested habitat in the absence of mammalian predators and competition from *L. americanus* (Small et al. 1992). *Lepus othus* may be able to similarly persist in forested habitat. In Newfoundland, *L. americanus* has been shown to be more resilient than *L. arcticus* to predation by Red Foxes (Small and Keith 1992), possibly by favoring understory cover. *Lepus arcticus* may also struggle to escape predators in the soft, deep snow in Newfoundland's shrub and forest habitat where *L. americanus* is more successful (Mercer et al. 1981). The more abundant prey species, *L. americanus*, may have caused a "high density of randomly foraging [shared] predators that 'spill over' into other habitats and decrease the abundance and

distribution of alternative prey," in this case, *L. arcticus* (Small and Keith 1992, p. 1620). Red Foxes have expanded their range in Alaska and other northern regions (Killengreen et al. 2007, Post et al. 2009), moving north of the Brooks Range in the 20th century but largely keeping to the foothills and river corridors except during occasional periods of abundance on the tundra plain of the North Slope (Savory et al. 2014). Red Foxes did become more common on the North Slope in the late 20th century, especially in association with oil development infrastructure, but *L. othus* persists in western Alaska in partial sympatry with *L. americanus* and *V. vulpes*, so the evidence does not support predator spillover as the cause for the potential disappearance of *L. othus* from north of the Brooks Range.

Zooarchaeological evidence suggests that *L. americanus* has replaced *L. othus* in the Lime Hills Cave region (Fig 1.3), located in what was formerly tundra/alpine habitat but is now boreal spruce-birch forest (Endacott 2008). Endacott (2008) identified all hare remains from the deepest soil strata (late Wisconsin-14,000 years ago) as *L. othus* and all hare remains from the top strata (≥8,000 years ago) as *L. americanus*. The middle strata (14,000-8000 years ago) contained remains of both species. This pattern has been interpreted as being "clearly the result of reduced tundra/alpine habitat in the area and expansion of shrubs and boreal forest" (Endacott 2008, p. 233), a shift in habitat similar to the present expansion of shrubs and trees into former arctic tundra (Hinzman et al. 2005), although on vastly different time scales. Archaeological remains identified as *L. othus* have been reported from interior Alaska (Dixon 1984; Weber et al. 1981), which is now predominantly boreal forest and well outside the current distribution of *L. othus*. Zooarchaeological and ethnological material could provide a better understanding of the historical distribution of *L. othus* and how it has changed over time. However, a combination of ancient DNA extracted from Beringian paleontological hare remains, together with orders of

magnitude more molecular markers than have been employed in the past will almost certainly be required for definitive resolution.

*Taxonomy and gene flow*.—Consideration of the Alaskan Hare's distribution requires an understanding of its tortuous taxonomic history and current status. *Lepus othus* is part of a species complex comprising the subgenus *Lepus* Linnaeus 1758, which currently includes two other northern hare species: the Arctic Hare (*L. arcticus*) and the Mountain Hare (*L. timidus*). As currently circumscribed taxonomically, *L. othus* is restricted to western Alaska, *L. arcticus* occurs in Greenland and northern Canada from the Northwest Territories to Newfoundland and Ellesmere Island, and *L. timidus* ranges from the Chukotsk Peninsula in far eastern Russia to eastern Poland, throughout Scandinavia, and in isolated populations in Japan, the British Isles, and the Alps (Angerbjörn and Flux 1995).

Geography and allopatry are the principal grounds upon which *L. othus*, *L. arcticus*, and *L. timidus* have retained their separate species status (Anderson 1974). Morphological (Baker et al. 1983; Dixon et al. 1983) and molecular (Waltari and Cook 2005; Alves et al. 2008; Melo-Ferreira et al. 2012) data have been inconclusive or conflicting, and the taxonomy of northern hares remains in dispute. Ranges of the three species are not thought to overlap and therefore gene flow is not thought to occur (Anderson 1974).

The identification (*L. othus* or *L. timidus*) of the northern hare sighted on St. Lawrence Island in winter 2012 is unclear. St. Lawrence is 75 km from the Chukotsk Peninsula but 190 km from the nearest Alaskan mainland, suggesting that the hare(s) in question likely originated from Russia. Thus, presumably infrequent but recurrent gene flow between the two species may be facilitated by island stepping stones, especially if their thick pelage is providing sufficient insulation to forgo foraging for up to 15 days, as has been theorized for *L. arcticus* (Wang et al.

1973). Wolverine (*Gulo gulo*) and Brown Bear (*Ursus arctos*) are also observed on St. Lawrence Island occasionally, although there are no sustaining populations of either species on the island (J. J. Burns, pers. comm.). However, as climate change continues to affect sea ice extent in the Arctic (Bernstein et al. 2007), there could be a reduction in potential gene flow between in Eurasia, Alaska, and the islands between the two continents.

Clarifying the distributional limits of *L. othus* will require resolution of northern hare taxonomy given the potential for gene flow between *L. timidus* from eastern Russia and *L. othus*. When the McIlhenny specimen was collected, it was originally identified as *Lepus tschuktschorum* Nordquist 1883, according to Stone (1900). *L. tschuktschorum* was a junior synonym of *L. othus* that included northern hares from Eastern Siberia and Alaska. If eastern Siberian hare populations are considered conspecific with *L. othus,* then "[*Lepus*] *tschuktschorum* Nordquist, 1883 has priority over [*Lepus*] *othus* Merriam, 1900" (Hoffmann and Smith 2005, p. 202).

It is widely agreed that high-latitude regions have been impacted by climate change in the past century and are likely to be inordinately affected by continuing changes in phenology, vegetation cover, snow cover, and ground icing conditions (Bernstein et al. 2007; Hinzman et al. 2005; Jorgenson et al. 2015; Prowse et al. 2009). These changes could make foraging more difficult for herbivores. Shrub cover in the Alaskan Arctic has increased over the past century and is expected to continue to expand, and treeline encroachment into formerly tree-less areas has been documented in western and northern Alaska (Hinzman et al. 2005; Prowse et al. 2009). Such conditions will likely favor the continued expansion of *L. americanus* into northern and western Alaska. Tundra-associated mammals such as the Barren Ground Shrew (*Sorex ugyunak*)

and Singing Vole (*Microtus miurus*) are predicted to undergo range shifts away from western and southern extents of arctic tundra (Baltensperger and Huettmann 2015; Hope et al. 2013).

Although the historical occurrence and abundance of *L. othus* on the North Slope remains unclear, a northern shift from its current range is predicted by Leach et al. (2015) and may be critical to its long-term viability. Most of the western North Slope lies within the National Petroleum Reserve - Alaska (NPR-A), federal land set aside for potential oil and gas operations. The U.S. Bureau of Land Management has noted the current presumed absence of *L. othus* in its environmental impact statement for the NPR-A (BLM 2012). However, by the end of the 21st century this region may be the only large expanse of continuous unforested habitat left in Alaska (Hope et al. 2013; Prowse et al. 2009) and much of the shifted range of *L. othus* as predicted by Leach et al. (2015) lies within the NPR-A, a prospect that echoes recent calls (reviewed by Hannah 2011) for considering probable future range shifts in conservation and management plans.

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# TABLES

Table 1.1—Names and abbreviations of museums and other collections searched through online databases, contact with curators and collection managers, and/or personal visits.

| Museum Name | Museum Abbreviation |
|---|---|
| Academy of Natural Sciences | ANSP |
| American Museum of Natural History | AMNH |
| Burke Museum of Natural History and Culture, University of Washington | UWBM |
| California Academy of Sciences | CAS |
| Canadian Museum of Nature | CMN |
| Carnegie Museum | CM |
| Charles R. Connor Museum | CRCM |
| Cornell University Museum of Vertebrates | CU |
| Cowan Vertebrate Museum, University of British Columbia | UBC |
| Denver Museum of Nature and Science | DMNS |
| Dickey Collection, University of California, Los Angeles | UCLA |
| Field Museum of Natural History | FMNH |
| Humboldt State University Wildlife Collection | HSUW |
| Izembek NWR | |
| James R. Slater Museum of Natural History, University of Puget Sound | PSM |
| Los Angeles County Museum | LACM |
| Louisiana State University, Museum of Natural Science | LSUMZ |
| Michigan State University Museum | MSU |
| Monte L. Bean Life Science Museum, Brigham Young University | BYU |
| Museum of Comparative Zoology, Harvard | MCZ |
| Museum of Southwestern Biology, University of New Mexico | MSB |
| Museum of Texas Tech University | TTU |
| Museum of Vertebrate Zoology, University of California | MVZ |
| New Mexico Museum of Natural History | NMMNH |
| New York State Museum | NYSM |
| Oklahoma Museum of Natural History, University of Oklahoma | OMNH |
| Royal British Columbia Museum | RBCM |
| Royal Ontario Museum | ROM |
| Texas Cooperative Wildlife Collection | TCWC |

Table 1.1 continued

| | |
|---|---|
| United States National Museum of Natural History (Smithsonian) | USNM |
| University of Alaska Museum | UAM |
| University of Alberta Museum of Zoology | UAMAZ |
| University of California Davis | UCD |
| University of Colorado Museum | UCM |
| University of Kansas, Natural History Museum and Biodiversity Center | KU |
| University of Michigan Museum of Zoology | UMMZ |
| University of Wisconsin Zoological Museum | UWZM |
| Utah Museum of Natural History, University of Utah | UMNH |
| Western New Mexico University | WNMU |

Table 1.2.—Environmental variables and their relative importance to the spatial distribution model.

| Variable | Relative Score |
|---|---|
| Distance to March sea ice | 100.00 |
| Distance to coastline | 68.22 |
| Cliome | 47.74 |
| Geology | 36.26 |
| Elevation | 28.30 |
| Mean temperature for September/October/November | 21.91 |
| Mean % of days where precipitation fell as snow in September/October/November | 10.21 |
| Mean first day of freeze | 8.36 |
| Mean % of days where precipitation fell as snow in March/April/May | 7.27 |
| Mean temperature for March/April/May | 5.47 |
| Mean % of days where precipitation fell as snow in December/January/February | 5.42 |
| Mean ground temperature | 5.03 |
| Mean temperature for December/January/February | 4.49 |
| Distance to September sea ice | 3.85 |
| Mean first day of thaw | 3.84 |
| Distance to lakes | 2.51 |
| Mean number of grow days | 2.02 |
| Mean temperature for June/July/August | 1.98 |
| Mean % of days where precipitation fell as snow in June/July/August | 1.57 |
| Mean precipitation for September/October/November | 1.22 |
| Mean precipitation for March/April/May | 1.11 |
| Mean precipitation for June/July/August | 0.96 |
| Mean precipitation for December/January/February | 0.76 |
| Mean active layer thickness | 0.74 |
| Distance to streams | 0.63 |
| Aspect | 0.45 |
| Distance to wetland vegetation | 0.41 |
| Slope | 0.23 |

FIGURES

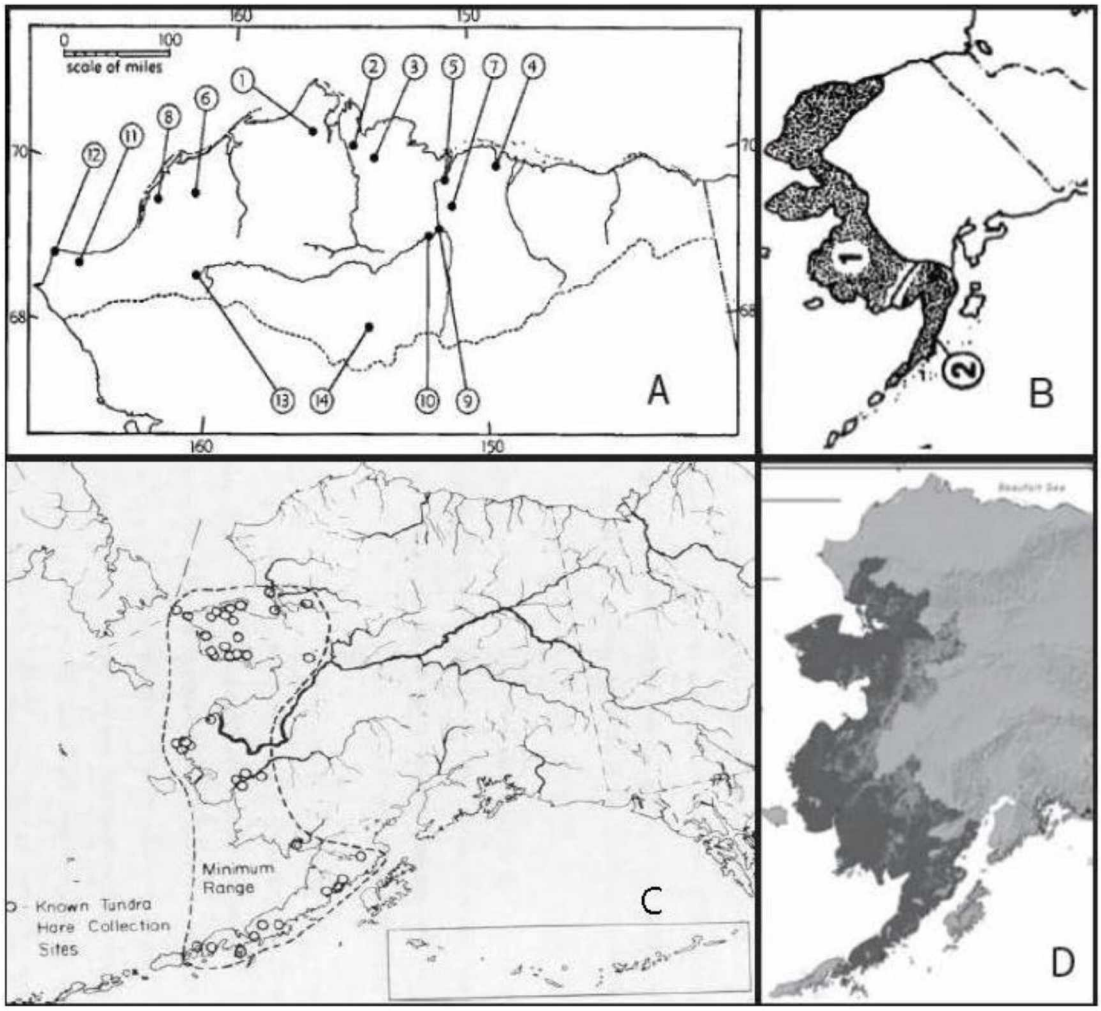


Figure 1.1—Previously published distribution maps for *Lepus othus*. A) North Slope distribution of *L. othus* from Bee and Hall (1956), B) Distribution from Best and Henry (1994) citing Hall (1981), C) Distribution from Anderson (1974), D) Distribution from Alaska Gap Analysis Project (http://aknhp.uaa.alaska.edu/zoology/akgap/).

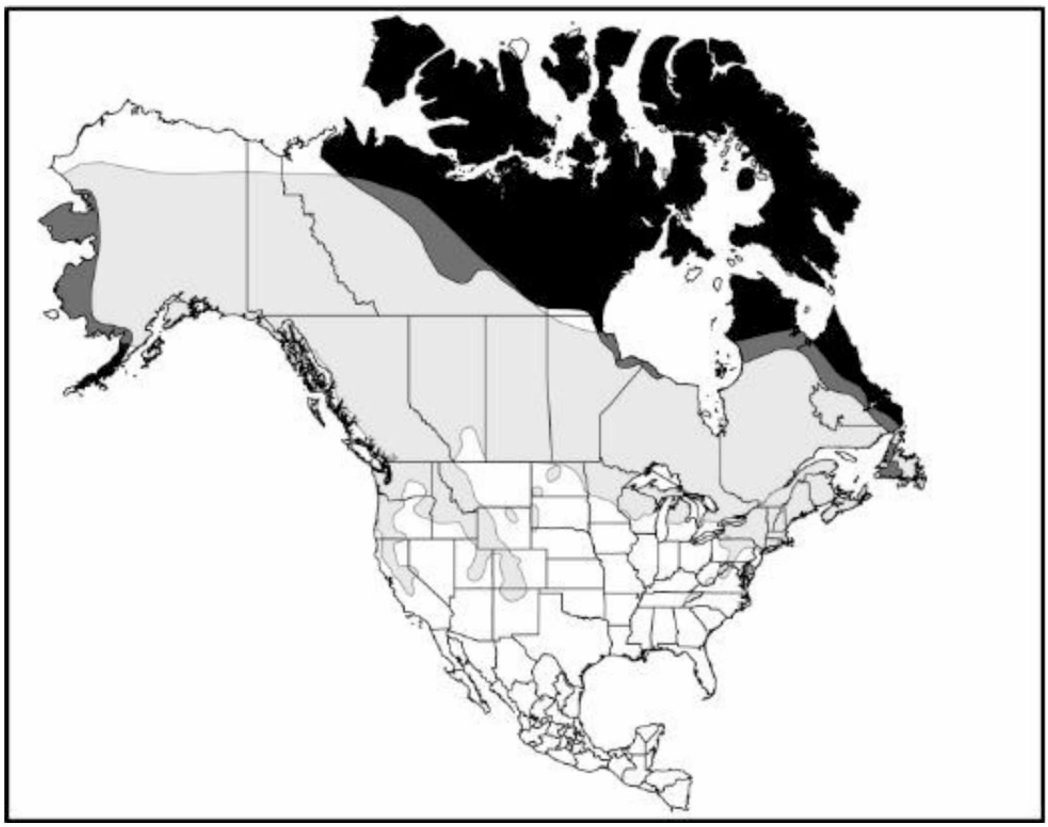

Figure 1.2.—IUCN range maps (Murray and Smith 2008a,b,c) of *Lepus othus* (black, western Alaska), *L. arcticus* (black, northern Canada), and *L. americanus* (light gray). Range overlap is shown in dark gray. The range of *L. arcticus* extends north and northeast off panel.

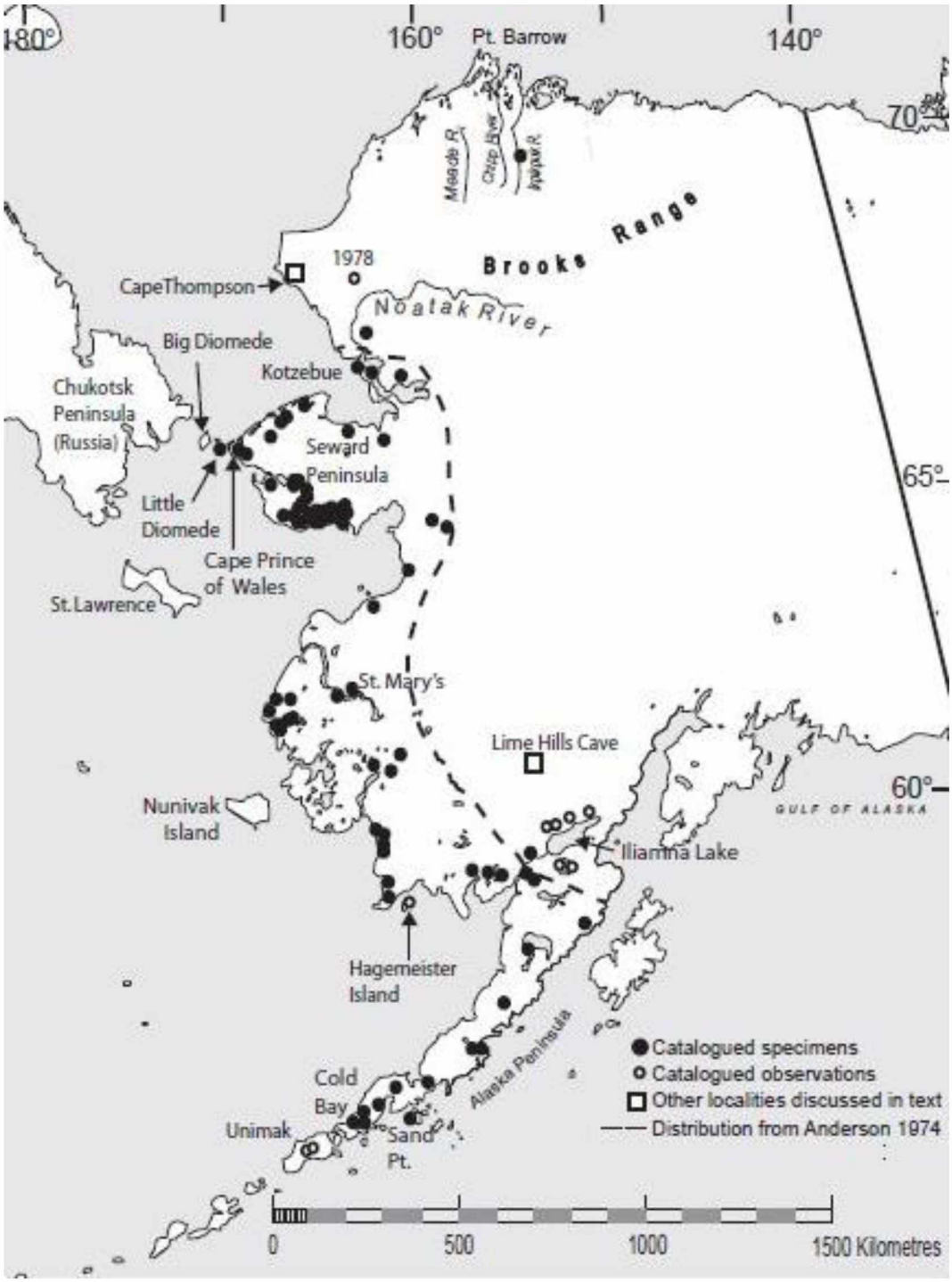


Figure 1.3.—Updated distribution map of *Lepus othus*: collection localities of all catalogued *L. othus* museum specimens and observations, other localities discussed in text, and the distribution boundary from Anderson 1974.

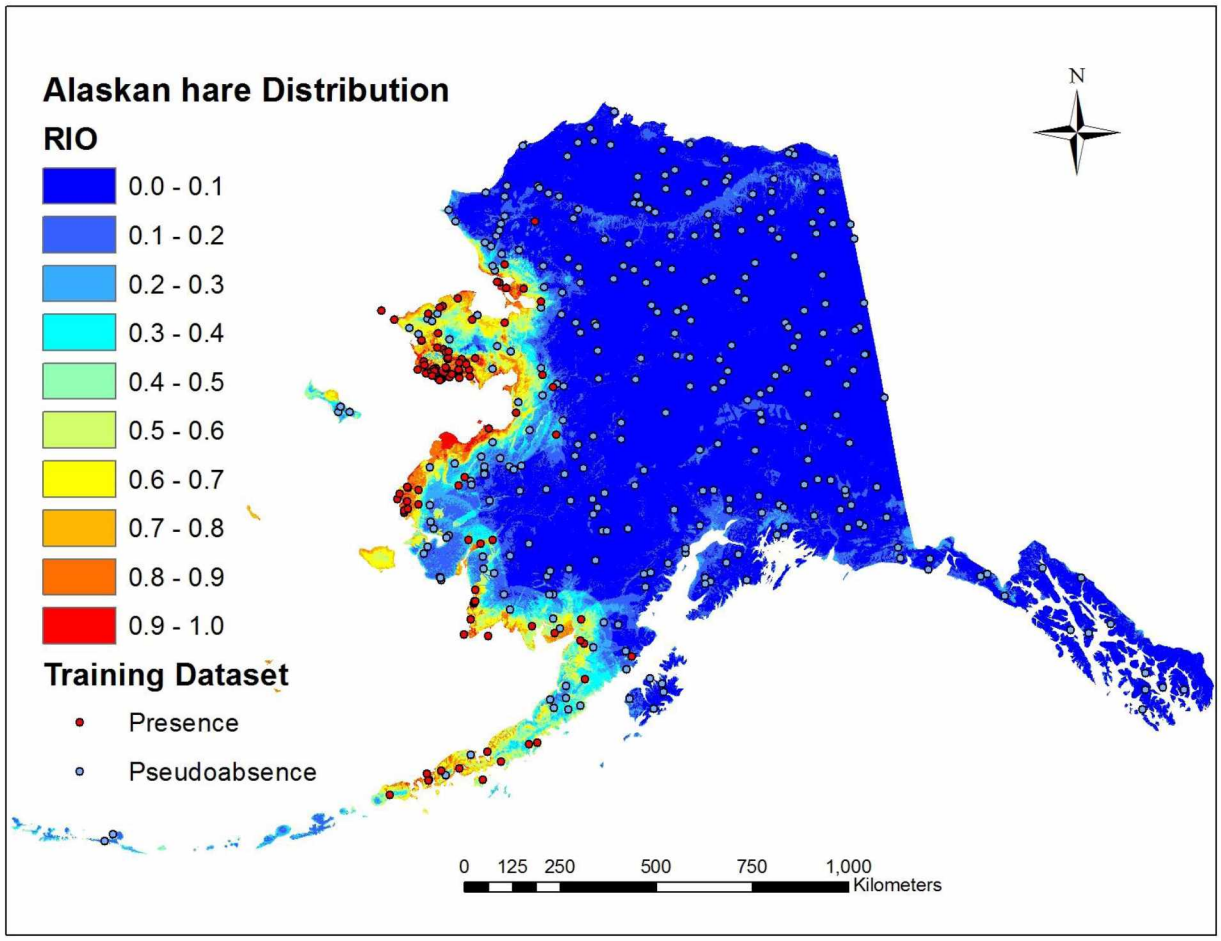


Figure 1.4.—Spatial distribution model of the present distribution of *Lepus othus*. Red circles mark presences (georeferenced collection localities). Blue circles indicate pseudoabsences. Relative indices of occurrence (RIO) values greater than 0.55 indicate regions of likely presence, and values less than 0.55 indicate regions of likely absence.

CHAPTER 2:

# Intraspecific mitochondrial DNA and morphometric variation in the endemic and taxonomically recalcitrant Alaskan Hare (*Lepus othus*) [2]


## ABSTRACT

The Alaskan Hare (*Lepus othus* Merriam 1900) is a charismatic Arctic mammal whose intraspecific variation has traditionally been attributed to differences between northern and southern subspecies. Alternatively, some researchers have proposed that the morphological variation reflects geographic variation across a latitudinal size cline. Recently collected specimens from regions in Alaska that were poorly represented in the past (i.e. Alaska Peninsula, Little Diomede, and Kotzebue Sound), allowed us to uncover more genetic diversity and population structure in the mitochondrial control region than was found in previous studies. Our morphological analyses of the difference between the two subspecies were ambiguous, with PCA and simple linear regression indicating the presence of a latitudinal size cline and DFA revealing successful group assignment that is not solely based on latitude. We also observed a mtDNA association with *L. timidus* Linnaeus 1758 in Eurasia, which, combined with new island specimens and observations, suggests gene flow across the Bering Strait. However, the species limits between *L. othus* and *L. timidus* remain murky, and will likely require genomic sequencing for clarification.


[2] Cason, M. M., K. M. Everson, D. E. Watts, T. L. Booms, and L. E. Olson. In prep. Intraspecific mitochondrial DNA and morphometric variation in the endemic and taxonomically recalcitrant Alaskan Hare (*Lepus othus*). Journal of Mammalogy.

# INTRODUCTION

The Alaskan Arctic is undergoing rapid climate change, imposing unique challenges on endemic species. Tundra habitat is expected to recede from southwest Alaska (Baltensperger and Huettmann 2015) and biome shifts are expected to occur on the Seward Peninsula within the next century (Murphy et al. 2010). Such environmental changes have the potential to alter phylogeographic relationships among northern taxa by decreasing and fragmenting tundra habitat and limiting gene flow. However, current phylogeographic relationships of even charismatic mammalian taxa are not always well documented in a region as remote and difficult to study as the Alaskan tundra, and changes in biodiversity may go undetected.

Unlike any other mammal species, the entire known distribution of the Alaskan Hare, *Lepus othus* Merriam 1900, is restricted to the coastal tundra of western Alaska (Cason et al. 2016), a region undergoing rapid environmental change (Murphy et al. 2010). The type locality is St. Michael, near Norton Sound (Hoffmann and Smith 2005a). Two subspecies are currently recognized: *L. o. othus* Merriam 1900 and *L. o. tschuktschorum* Nordquist 1883 (the latter is classified as *L. o. poadromus* Merriam 1900 by some authors; Hall 1981; MacDonald and Cook 2009). Despite this taxonomic subdivision, the Alaskan Hare is reported to contain relatively low levels of genetic diversity (Waltari and Cook 2005) and the morphological basis of the subspecies description has been attributed to clinal variation (Anderson 1974).

Its phylogenetic relationship to, and taxonomic distinction from, 2 closely related taxa—the Mountain Hare, *Lepus timidus* Linnaeus 1758, and the Arctic Hare *Lepus arcticus* Ross 1819—has also proven difficult to resolve (Baker et al. 1983; Melo-Ferreira et al. 2012). These three putative species form a species complex and cannot be distinguished from each other using morphology alone. Currently, geography is the only way to differentiate the 3 taxa. The

distribution of *L. timidus* stretches across the tundra and boreal forest of Russia, Fenno-Scandinavia, eastern Europe, the Alps, and the British Isles (Angerbjörn and Flux 1995). The type locality is Uppsala, Sweeden (Hoffmann and Smith 2005b). *L. arcticus* occurs in northern Canada and Greendland (Hall 1981), and the type locality is Bylot Island, Canada (Hoffmann and Smith 2005c). The terms 'arctic hare' and 'northern hare' have been used interchangeably to collectively refer to all 3 putative species; to avoid confusion, we adopt the use of 'northern hare' hereafter.

*Taxonomic history*.—In 1885, Murdoch identified the "polar hares" in northern Alaska as *Lepus timidus arcticus*, a name encompassing the northern hares in Arctic Canada and Greenland, and at the species level, included northern hares in Eurasia. Nordquist (1886) gave the name *L. tschuktschorum* to northern hares of northeastern Siberia, and in 1896, Rhoads synonomized Alaskan Hares with *L. tschuktschorum*.

Merriam (1900) described 2 species of large-bodied northern hare in Alaska: *L. othus*, the tundra polar hare, and *L. poadromus,* the peninsula arctic hare. In his revision of North American hares, Howell (1936) synonymized these species, demoting them to subspecies of *L. othus*: *L. o. othus* and *L. o. poadromus*. Howell stated based his subspecies differentiation on differences in cranial sizes and summer pelage coloration. He did not explicitly state his reasons for synonymizing *L. poadromus* with *L. othus* or for designating them as separate subspecies with non-overlapping geographic distributions. The range of *L. o. othus*, as described by Howell (1936:333), includes the "tundras of northern and northwestern Alaska, exclusive of the [Alaska] Peninsula and Bristol Bay section," while the distribution of *L. o. poadromus* was said to be restricted to the Alaska Peninsula and the Bristol Bay region. *L. othus* was judged distinct from *L. arcticus*, while *L. othus* and *L. timidus* were hypothesized to be "closely related" (Howell

1936:333) but nonetheless distinct, although Howell himself acknowledged that the relative paucity of comparative material available for *L. timidus* limited his ability to differentiate the two.

Hall (1951; 1981) concluded that *L. othus* should be synonymized with *L. timidus*—an arrangement later recognized by Rausch (1963)—but made no conclusions about the validity of *L. arcticus*. However, Hall (1951;1981) recognized two *L. othus* subspecies: *L. o. othus* and *L. o. poadromus*. He did not give his reasoning for recognizing subspecies of *L. othus*, or for concluding *L. othus* should be synonymized with *L. timidus*.

In the most comprehensive review of the Alaskan Hare to date, Anderson (1974) recognized 3 distinct species of worldwide northern hares based on skull measurements and geography: *L. othus*, *L. arcticus*, and *L. timidus*. Because *L. timidus* is found at similar latitudes as *L. othus*, but the range of *L. arcticus* extends farther north, Anderson predicted a size cline if the 3 are conspecific, with *L. timidus* similar in cranial dimensions to *L. othus* and *L. arcticus* surpassing both. However, he found *L. othus* to have larger skull and body measurements than either *L. timidus* or *L. arcticus* and concluded this lack of a size cline was additional evidence against conspecificity. He also determined that instead of falling into 1 of 2 subspecies, skull measurements of *L. othus* specimens exhibited a latitudinal size cline following Bergmann's Rule. Finally, Anderson cited geographic isolation and the presumed commensurate absence of gene flow as further support for recognizing *L. othus*.

Subsequent morphological investigations supported synonymization of 1 or more northern hares. Principal components analysis (PCA) of craniodental measurements from *L. othus*, *L. arcticus*, and *L. timidus* led Baker et al. (1983) to recognize all populations from Greenland, Canada, Alaska, and Russia's Chukotsk Peninsula as *L. arcticus* and those from the

rest of Eurasia as *L. timidus*. Their multivariate analyses suggested that the single specimen from the Chukotsk Peninsula was phenetically more similar to hares in Alaska as well as those from Banks and Prince Patric Islands, Canada. Based on PCA and cluster analysis, Dixon et al. (1983) concluded that the 3 northern hare taxa were conspecific and likely represented a circumpolar ring species under the name of *L. timidus*.

Recent studies based on molecular data have been similarily inconclusive. Waltari et al. (2004) recognized *L. othus*, *L. arcticus*, and *L. timidus* as distinct species based on DNA sequences from the mitochondrial control region. A clade containing all *L. othus* haplotypes and one *L. timidus* specimen from the Omolon River, Russia, was more strongly supported (0.81 Bayesian posterior probability) than was a clade with only the *L. othus* haplotypes (0.70 posterior probability). However, likelihood-ratio tests did not reject the monophyly of each species, and since other specimens from the Omolon River grouped with *L. timidus* clades and hares in that region are often found in forested instead of tundra habitat, Waltari et al. (2004) advocated for additional sampling from Beringia before revising the taxonomy of the 3 northern hare species. Waltari and Cook (2005) later added mitochondrial control region sequences from 97 additional specimens and found *L. othus* to be monophyletic, which they concluded verified its rank as a species (Waltari and Cook 2005). However, Waltari and Cook used only 7 (out of 61) *L. othus* specimens from outside of the Seward Peninsula. These 7 specimens were collected from 4 different localities, and only 1 was collected from the Alaska Peninsula (Cold Bay). Alves et al. (2006) criticized Waltari and Cook (2005) for relying solely on mitochondrial DNA, citing possible introgression (a well-documented phenomenon in other species of hares; Melo-Ferreira et al. 2009, 2012), hybridization, and pseudogene insertion into the nuclear genome.

Melo-Ferreira et al. (2012) sequenced nuclear and mitochondrial loci from *L. othus*, *L. arcticus*, and *L. timidus*. Their species trees and mtDNA-based phylogenies resulted in a northern hare polytomy, and an isolation-with-migration model did not support gene flow between any of the 3 species after speciation. Therefore, they did not synonymize the 3 northern hare species. Although northern hare taxonomy remains uncertain, *L. othus*, *L. arcticus*, and *L. timidus* are tentatively recognized as distinct species (Hoffmann and Smith 2005a, Murray and Smith 2008) until more definitive results indicate otherwise. An understanding of the taxonomic history of the northern hare species complex is necessary to place this investigation of intraspecific variation in context. However, determining the species limits among the 3 putative northern hare species is beyond the scope of this research.

Of the 3 northern hare species, *L. othus* has the smallest range and does not seem to have been widespread north of the Brooks Range, Alaska, in the past 130 years (Fig. 2.1; Cason et al. 2016). However, recent specimens deposited in the mammal collection at the University of Alaska Museum (UAM) (catalog numbers UAM 120797 and 122839) obtained from Little Diomede Island suggest that northern hares may be crossing sea ice between Eurasia and North America and using islands in the Bering sea as stepping stones (Cason et al. 2016). Because geography is currently the only way to differentiate between *L. othus* and *L. timidus*, and because the continental origin of these specimens is unknown, the species identification of these specimens is unclear.

Over the past half century, 11 studies (Bee and Hall 1956, Baker et al. 1978; Hall 1981; Flux 1983; Halanych et al. 1999; Waltari et al. 2004; Hoffmann and Smith 2005a, Waltari and Cook 2005; Alves et al. 2006; Murray and Smith 2008, Melo-Ferreira et al. 2012) have concluded that the northern hare species complex requires additional taxonomic research before

the relationship between the 3 species can be resolved. Alaska's Comprehensive Wildlife Conservation Strategy (Alaska Department of Fish & Game 2006) lists *L. othus*—1 of only 2 mammal species endemic to mainland Alaska—as a Species of Greatest Conservation Concern, largely due to the long-standing taxonomic confusion. In light of persistent taxonomic uncertainty at and below the species level and growing scientific consensus that the western Alaska tundra—encompassing the entirety of the Alaskan Hare's range (Cason et al. 2016)—is undergoing profound and rapid change, we address several lingering issues surrounding the circumscription and geographic distribution of this taxonomically recalcitrant Alaskan endemic.

We evaluated phylogeographic structure within *L. othus* and expanded Anderson's (1974) craniometrics dataset to test subspecies boundaries with specimens collected since his seminal study. Importantly, these include specimens from regions in Alaska that were poorly represented in the past (i.e. Alaska Peninsula, Little Diomede Island, and Kotzebue Sound). Tundra and Arctic grassland habitat has decreased in western Alaska since the end of the Pleistocene, and boreal forest spread from interior to western Alaska by the mid-Holocene, around 5,000 years ago (Anger 2013). Therefore, we predicted low genetic diversity and evidence of a decreasing effective population size of *L. othus*, corresponding with increasing forestation in Alaska since the Last Glacial Maximum. We also hypothesized that *L. othus* skulls would follow a latitudinal size cline rather than group according to recognized subspecies (Anderson 1974). We did not attempt to resolve the question of species status for the three members of the northern hare complex.

## MATERIALS AND METHODS

*Molecular methods.*— We sequenced 671 base pairs of mitochondrial DNA (472 bp from the control region, 65 bp from the upstream tRNA-Pro locus, 67 bp from the tRNA-Thr locus,

and 65 bp from cytochrome-*b*) from 63 *L. othus* specimens archived in the Mammal Collection at UAM (catalog numbers are provided in Supplemental File Appendix 2.1). Genomic DNA was extracted from fresh or frozen tissue samples using the PureGene Genomic DNA Purification Kit (Gentra Systems, Minneapolis, Minnesota). Standard PCR amplifications were conducted using primers LEPUS3' (Waltari et al. 2004) and TDKD (Slade et al. 1994). PCR products were purified using Exo-Sap$^{TM}$ (USB Corp., Cleveland, Ohio) and bidirectionally sequenced at the University of Washington's High Throughput Genomics Center.

Sequences were assembled and aligned by eye in Sequencher ver. 5.2.3 (Gene Codes, Ann Arbor, MI) with reference to a *L. othus* partial mitochondrial genome obtained from GenBank (KJ397608.1). Our phylogenetic analyses included an additional 56 *L. othus*, 56 *L. arcticus*, and 61 *L. timidus* mtDNA control region sequences received from Waltari and Cook's (2005) study provided by E. Waltari. There were no insertions or deletions in the alignment of the 3 northern hare species.

*Molecular analyses.*—We used PopArt ver. 1.7 (http://popart.otago.ac.nz) to produce a median-joining haplotype network (Clement et al. 2002) of the northern hare species complex. We used Arlequin ver. 3.5.1.2 (Excoffier and Lischer 2010) to investigate population structure with analyses of molecular variance (AMOVA) and pairwise population differentiation. *L. othus* samples were grouped into populations separated by at least 200 km, the maximum recorded dispersal distance of a northern hare (Angerbjörn and Flux 1995). Six such populations (Alaska Peninsula, Bristol Bay, Kuskokwim Delta, Yukon Delta, Seward Peninsula, and Little Diomede; Fig. 2.2) were considered in different AMOVA groupings. We also used Arlequin to calculate standard molecular diversity indices and test for historical changes in population size with Tajima's $D$ (Tajima 1989), Fu's $F_S$ (Fu 1997), and mismatch distributions (Slatkin and Hudson

1991; Rogers and Harpending 1992). For populations whose mismatch distribution did not significantly differ from the sudden-expansion model (Rogers 1995) we estimated the amount of time since demographic expansion with the equation $\tau=2\mu t$. We used a mutation rate of 12.4% per site per million years, which is the published mutation rate for *Lepus* mtDNA control region (Pierpaoli et al. 1999). With a 482 bp control region and a 2 year generation length (Pierpaoli et al. 1999), the value of $\mu$ is 0.00012. The amount of time since the population expansion (in generations) is $t$, and $\tau$ approximates the mode of the mismatch distribution, which is an index of the amount of time since population expansion (Slatkin and Hudson 1991).

We used jModelTest ver. 2.1.7 (Posada 2008) to determine the best-fit model of DNA substitution and we constructed a Bayesian skyline plots (BSP) in BEAST ver. 1.8.1 (Drummond et al. 2014) to examine population fluctuations over time with a coalescent-based approach. The same mutation rate of 12.4% per million years (Pierpaoli et al. 1999; Waltari et al. 2004) was used, the MCMC chain length was 100,000,000 generations, trees and parameters were sampled every 1000 generations, and the initial 10% of the output was removed as burn-in. We used Tracer v. 1.5 (Rambaut et al. 2009) to construct BSP intervals.

*Morphological analyses.*— Twenty-four measurements were recorded from 94 *L. othus* skulls to the nearest 0.01 mm using digital calipers. All measurements were taken by the first author and are described in Anderson (1974; Table 2.5). Our measurements were compiled with those from 161 specimens measured and reported by Anderson (1974). We tested the repeatability of our measurements with the Intraclass Correlation Coefficient (ICC; Lessells and Boag 1987; Klaczko et al. 2015). We measured 5 specimens (UAM 18476, 18501, 18502, 18505, 18508) 10 times each and generated an ICC score with the ICC package (Wolak et al.

2012) in the program R (R Core Team 2014). We considered measurements with ICC scores greater than 0.9 to be repeatable.

The 5 specimens that we measured 10 times (for repeatability test, above) were skulls that Anderson (1974) had also measured. We evaluated the reproducibility of each measurement trait with a Wilcoxon rank sum test, which is a nonparametric, unpaired version of a two-sampled *t*-test. We excluded measurements with *P*-values that indicated a significant (at the 0.05 level) difference between our data and Anderson's to ensure that we only used highly reproducible measurements.

To investigate potential sexual dimorphism we compared measurements of 30 males and 21 females (Supplemental File Appendix 2.2) from a single locality and season (Arctic River 1971-1972) with a Student's t-test. Specimens were aged according to femoral epiphyseal closures and, when femurs were not available, the degree of development of the anterior supraorbital process of the skull. Both of these methods have been used to age hares (Manning and Macpherson 1958; Lechleitner 1959; Anderson 1974). Our age determinations are included in Appendix 2.2. We did not address collection year as a factor of body size in our morphological analysis.

We used Principal Component Analysis (PCA), Discriminant Function Analysis (DFA), and simple linear regression in JMP® Pro ver. 11.1.1. (SAS institute Inc., Carey, NC.) to investigate variation in skull measurements within *L. othus* and between purported subspecies *L. o. othus* and *L. o. tschuktschorum*, called *L. o. poadromus* by Hall (1981) whose map of the subspecies boundary we use. Data were normalized by taking the base 10 logarithm of all measurements. Principal components were analyzed on covariances using the restricted maximum likelihood estimation method. Using DFA, we divided specimens by subspecies and

then, alternatively, grouped specimens arbitrarily by latitude to test if differences in skull measurements are due to a latitudinal size cline instead of a subspecies distinction. Scenario 1, the current subspecies distinction according to (Hall 1981), separates the 2 subspecies around Togiak Bay (Fig. 2.3), which splits the subspecies at 59°N with 3 exceptions: UAM 119025 was collected at latitude 58.6476°N but is on the *L. o. othus* side of the subspecies division, UAM 118123 was collected at latitude 59.2202°N but is on the *L. o. poadromus* side of the subspecies division, and UAM 97801 was collected at latitude 59°N on the *L. o. poadromus* side. In scenario 2 we separated all *L. othus* specimens in 2 groups at 57°N, in scenario 3 we separated the 2 groups at 61°N, and in scenario 4 we separate the 2 groups at 64°N. We also plotted each measurement against latitude to investigate a possible latitudinal size cline.

## RESULTS

*Haplotype network.—Lepus othus* falls into 3 haplogroups in the median joining network (Fig. 2.4a-c). The largest haplogroup contained 108 of the 119 *L. othus* specimens. All of the specimens from the Seward Peninsula, Yukon Delta, and Kuskokwim Delta populations were recovered in this haplogroup. 3 of the 5 specimens from the Bristol Bay population and 2 of the 10 specimens from the Alaska Peninsula population were also in this cluster. The remaining 10 specimens from Bristol Bay and the Alaska Peninsula were in a haplogroup that was more genetically similar to *L. timidus* haplotypes than to the rest of the *L. othus* haplotypes. One Little Diomede specimen was equally similar (3 base pairs different) to the other specimen from Little Diomede and to a *L. timidus* specimen from Magadan, Russia (GenBank accession DQ067371). Other *L. timidus* haplotypes that were similar to the Little Diomede specimens were collected from Chita, Russia (7 and 8 base pairs different) and the Omolon River (8 and 9 base pairs

different). The *L. othus* haplotypes (from Noorvik, and Noatak) most similar to the Little Diomede specimens were 11 and 12 base pairs different, respectively.

*Population differentiation and molecular diversity.*—The $\Phi_{ST}$ value for *L. othus* as a single group was high (0.69), indicating population structure is present in the species. We compared AMOVA results of *L. othus* populations grouped by subspecies to all other possible groupings of the 6 populations. The variance between subspecies relative to the total variance ($\Phi_{CT}$) and variance among populations relative to the total variance ($\Phi_{ST}$) were both high (0.54 and 0.78 respectively; Table 2.1), indicating both of those groupings explain much of the variance present in *L. othus* as a putative species. However, an AMOVA with the Alaska Peninsula population in 1 group and all other *L. othus* populations in another group yielded higher $\Phi_{CT}$ (0.59) and $\Phi_{ST}$ (0.82) values than did the AMOVA with 2 groups aligned by subspecies. An AMOVA with the Kuskokwim Delta population and the Yukon Delta population in 1 group and the remaining 4 populations grouped individually had the highest $\Phi_{CT}$ value (0.70) and a high $\Phi_{ST}$ value (0.69).

Populations were compared with pairwise $\Phi_{ST}$ and average pairwise differences (Table 2.2) with 1000 permutations and assuming a Tamura and Nei (Tamura and Nei 1993) model of sequence evolution. Values of both indicators were low between Kuskokwim Delta and Yukon Delta populations, indicating there is little genetic difference between the 2. Therefore, we have combined these 2 populations into 1, hereafter referred to as the Y-K Delta population. The $\Phi_{ST}$ values were high and significant for all other population combinations except for the Bristol Bay population compared to the Alaska Peninsula, Kuskokwim Delta, and Little Diomede populations. The Little Diomede and Alaska Peninsula populations had high and significant values of average pairwise differences compared to all populations except for Bristol Bay. The

Bristol Bay population has moderate but significant values compared to the Seward Peninsula population, but non-significant moderate and low levels of average pairwise differences compared to all other populations.

The Bristol Bay *L. othus* population had the highest levels of genetic diversity as measured by the number of segregating sites ($S$), haplotype diversity ($H_d$), per-site nuclear diversity ($\pi$), and mean number of pairwise differences ($d_x$; Table 2.3). The Alaska Peninsula population also had relatively high levels of all measurements of genetic diversity.

*Historical demography.*—Tajima's $D$ (Tajima 1989) statistic was insignificant for all populations (Table 2.3). The Seward Peninsula was the only population with a significant ($p$=0.017) value for Fu's $F_S$ statistic (Fu 1997). The negative value (-5.418) indicates recent population expansion in the Seward Peninsula. The mismatch distributions for Bristol Bay and Y-K Delta populations differed significantly from the sudden-expansion model (Table 2.4). Mismatch distributions for all other populations did not significantly differ from the sudden-expansion model. Therefore, we calculated the number of years since population expansion from the Alaska Peninsula (0 years), Seward Peninsula (17,944), and *L. othus* as a whole (240,396). However, the confidence interval for the $\tau$ value (28.736) of the whole species of *L. othus* was extremely wide (0.00, 113.736). The BSP for the species of *L. othus* provides little support for demographic change over time (Fig. 2.5). The trend of the median population size for *L. othus* suggests a slight recent demographic decline, but that trend is not maintained in the upper 95% confidence intervals.

*Morphological analyses.*— Eleven of the 24 traits had very high ICC scores (>0.9) and were considered repeatable (Table 2.5). These traits also had low variation (<0.2) between repetitions of the same measurement for the same specimen (within group variance). According

to the Wilcoxon's rank sum tests, there was no significant difference between our measurements for the 11 repeatable traits and those measured by Anderson (1974; Table 2.5), and our measurements were therefore pooled with his.

We found no sexual dimorphism, which is consistent with previous research on *L. othus* (Anderson 1974) and *L. arcticus* (Manning and Macpherson 1958; Baker et al. 1978). Specimens that were determined to be juveniles or could not be confidently aged were excluded from analysis. Two specimens (USNM 6120 and 8645) were excluded because the error associated with their geographic coordinates was greater than 200 km (see Ch. 1). After excluding juveniles and specimens of indeterminate age as well as those with excessive error radii, our dataset included 203 specimens.

There is overlap between the 2 subspecies in the PCA plot of the first 2 components (Fig. 2.6), which account for 70.319% of the variance (Table 2.6). The matrix of loading scores (Table 2.7) shows the contribution from each variable to each factor. All variables (skull measurements) have a moderate to high (>0.5) contribution to the first component. In the second component, length of the palatal bridge has a negative loading value, contrasting with all other loading values for that component, which have low or moderately positive scores.

DFA correctly identifies all but 2 specimens to subspecies. These specimens (UAM 62589 from the Yukon Delta and UAM 66339 from Nome) were identified as *L. o. othus* but were collected in the geographic range of *L. o. tschuktschorum*. However, there is overlap in the canonical plot (Fig. 2.7a). The difference in skull measurements may be due to a latitudinal size cline instead of a subspecies distinction, and DFA could be distinguishing between the 2 groups because individuals tend to be larger as latitude increases and subspecies have traditionally been defined based on latitude (Hall 1981). Therefore, we produced a series of DFA plots of different

2-group assemblages based on latitude (Figs. 2.7b-d). DFA for scenario 2 (separation of 2 groups at 57°N; Fig. 2.7b) also identified all but 2 specimens to the correct north/south group. Scenario 3 (separation at 61°N; Fig. 2.7c) misclassified 16 specimens: 8 specimens in each group were classified as belonging to the other. Scenario 4 (separation at 64°N; Fig. 2.7d) misclassified 19 specimens: 8 specimens belonging to the northern group were classified as southern, and 11 specimens belonging to the southern group were classified as northern.

The simple linear regression for each measurement over latitude had a positive slope (Table 2.5). However, each slope value was low because the data were log transformed. The amount of variance explained by the models ($R^2$ values) was low to moderate (under 0.4; Table 2.5).

## DISCUSSION

*Molecular analysis.*— Paired $\Phi_{ST}$ and corrected average pairwise differences show the Little Diomede and Alaska Peninsula populations are distinct from all other populations except Bristol Bay. The Yukon and Kuskokwim Delta populations show little difference from each other, and we considered them a single population in subsequent analyses.

These inter- and intra-population measurements fit with a scenario of 2 distinct periods of immigration of northern hares into Alaska during the Quaternary: the first one establishing a refugium for *L. othus* in eastern Beringia during glacial advances (Waltari and Cook 2005), and the second wave bringing haplotypes that are more closely associated to modern *L. timidus* to the Alaska Peninsula. Waltari and Cook (2005) postulated gene flow between west and east Beringian northern hares but the flooding of the Bering Land Bridge in the Holocene led to a vicariance event and the divergence of the 3 currently recognized northern hare species. However, they acknowledge that "the number of colonizations across the Bering Strait region is

also equivocal" (Waltari and Cook 2005:3010), referring to the possibility of multiple founding events that led to *L. othus* and more than one refugial populations of *L. arcticus*. With the 2 distinct *L. othus* haplogroups, it now seems likely that there were multiple colonizations or introgression events in the evolutionary history of *L. othus*. Reconstructions of glacial extent in the late Wisconsin period (24,000-12,000 years ago) show an ice-free sliver of what is now the northern Alaska Peninsula on the Bristol Bay coast (Kaufman et al. 2011). The locations of the 2 distinct haplogroups are congruent with a hypothesis of northern hares following the retreat of the Cordilleran ice sheet from what is now the Bristol Bay region into the Alaska Peninsula as more habitat became available. However, we cannot say if those dispersers were recent arrivals from western Beringia or from a geographically expanding northern hare population in Alaska. The prevalence of the Alaska Peninsula haplogroups in southwestern Alaska may be due to immigration or introgression from western Beringia, from mitochondrial sweep of dispersers as land ice retreated, from a mitochondrial sweep that has occurred during the Holocene, or from incomplete lineage sorting.

Typical post-Pleistocene expansion often leaves a pattern of high genetic diversity in areas that were refugia and progressively less diversity following paths of expansion (Provan and Bennett 2008). However, zones of secondary contact between previously sundered lineages can have high levels of genetic diversity and can erroneously appear to represent refugia (Hewitt 2000). We found Bristol Bay and the Alaska Peninsula to have the highest levels of mitochondrial diversity of the mainland populations because both distant haplogroups are represented: the main *L. othus* haplogroup, and the Alaska Peninsula haplogroup, which is more similar to *L. timidus*. Because the 2 haplogroups are sympatric in Bristol Bay, it has the highest

levels of mitochondrial diversity, with haplotypes that are also found toward the terminus of the Alaska Peninsula and haplotypes that are found in the Y-K Delta.

Interestingly, the Little Diomede samples are most similar to a *L. timidus* haplotype from Magadan, Russia, over 2,000 km to the southwest. The *L. timidus* collection locality that is geographically closest to Little Diomede Island is about 550 km away, on the Chukotka Peninsula. However, only one *L. timidus* specimen from far eastern Russia has a similar haplotype to the Little Diomede specimens. It was collected at the Omolon River, over 1000 km away.

Little Diomede Island is less than 8 $km^2$ and because of the harsh Bering Strait climate, vegetation is sparse. Subsistence hunting and whaling are the primary food sources for locals, who are very familiar with the island's wildlife. There is no sustaining population of northern hares on Little Diomede Island, but according to local sources (Gay G. Sheffield, [University of Alaska Fairbanks, Fairbanks, AK], personal communication, [July 2014]), the specimens included in this study were part of a group of northern hares that arrived on the island in June 2014. Little Diomede Island (U.S.A) is less than 4 km from Big Diomede Island (Russia) and groups of northern hares have been observed on the sea ice off the western coast of the Seward Peninsula. Although communication between Little and Big Diomede is limited, there are accounts of northern hares on Big Diomede (John J. Burns, [retired, Alaska Department of Fish and Game, Fairbanks, AK], personal communication, [May 2015]). It seems likely that the specimens from Little Diomede dispersed from Big Diomede. Mainland Russia and Alaska are less than 50 and 40 km away from Little Diomede, respectively, and it is also possible the two specimens in this study dispersed from either side of the Bering Strait. However, because the haplotypes most similar to those from Little Diomede are not from either the Chukotsk or

Seward Peninsulas, it is also possible there is a population of northern hares on Big Diomede that does not have mitochondrial sequences similar to its nearest geographic neighbors.

Although haplotypes from Little Diomede, the Alaska Peninsula, and Bristol Bay are similar to *L. timidus* haplotypes, it is unclear how much gene flow historically occurred and is still occurring. Mitochondrial introgression among *L. timidus* and several European hare species (*L. europaeus*, *L. granatensis*, *and L. castrovejoi*) has been documented, leading to conflicting mitochondrial and nuclear phylogenies (Melo-Ferreira et al. 2009, 2012). Competitive replacement with hybridization (Melo-Ferreira et al. 2007, Acevedo et al. 2015) and adaptive evolution of mtDNA (Melo-Ferreira et al. 2009, 2014) have been proposed as drivers of introgression of *L. timidus* mtDNA into temperate congeners. Other mammalian taxa have been documented displaying similar disjunction between mitochondrial and nuclear phylogenies due to introgression (Good et al. 2015).

Notably, the *L. arcticus* haplotypes do not show the same gene flow with the other 2 species that *L. othus* displays with *L. timidus*, likely due to the allopatric distributions of *L. othus* and *L. arcticus*, with neither species occupying Alaska's North Slope. The reason for the lack of northern hares on the tundra of the North Slope and far western Canada remains a mystery.

The historical demographic analyses of *L. othus* as a whole are inconclusive, with either non-significant results (Tajima's $D$ and Fu's $F_S$) or with extremely wide confidence intervals (mismatch distribution and BSP). However, the mismatch distribution suggests the Seward Peninsula population experienced demographic expansion in the late Pleistocene (around 89,000 years ago), after the last interglacial but before the last glacial maximum. The significant Fu's $F_S$ value for the Seward Peninsula population also suggests a demographic expansion. The mismatch distribution for the Alaska Peninsula population also indicates there has been a recent

demographic expansion, but calculations with the $\tau$ value suggest the expansion is ongoing. Tajima's $D$ and Fu's $F_S$ values for the Alaska Peninsula population are not significant.

*Morphological analysis.*—The morphological results were ambiguous - the differences in skull measurements among northern and southern specimens were not completely explained by latitude. Although there was noticeable overlap in the PCA plot, and the linear regression showed each measurement increased with latitude, DFA correctly identifies all but 2 specimens by subspecies. DFA identifications by north/south group progressively worsen in the subsequent scenarios when the division is moved north, indicating clinal variation may not underlie subspecies boundaries. However, the DFA with specimens divided into 2 groups at 57°N, comparing specimens from the southern half of the Alaska Peninsula to those from the rest of western Alaska, performs equally well. This reveals that although there may be a morphological separation between northern and southern *L. othus* specimens, the location of the separation may not necessarily correspond with the current subspecies boundary.

*Conclusions.*— The separate haplogroups within *L. othus* indicate there may have been 2 distinct colonization events of northern hares in Alaska, or introgression from *L. timidus* and a mitochondrial sweep that has been restricted to the Alaska Peninsula and Bristol Bay area. The subspecies description, based on craniodental morphology, corresponds with the geographic locations of each haplogroup. However, our own morphological analyses were ambiguous, with PCA and simple linear regression indicating the presence of a latitudinal size cline and DFA revealing successful group assignment that is not solely attributable with latitude. Nevertheless, our morphological results do not contradict the possibility of a contact zone between the northern and southern haplogroups.

It seems premature to classify northern hares in Alaska to subspecies until we have better resolution of the species limits among *L. othus*, *L. timidus*, and *L. arcticus*. While there may indeed be a distinction between hares in southwest Alaska compared to the rest of western Alaska, it is unclear how much genetic difference there is genome-wide and if the distinction warrants subspecies status. In other words, "subspecies can't be known until the species limits are known" (Winston 1999: 324), and the non-geographic species limits of the 3 northern hare species remain indistinct. Additionally, an accurate subspecies description involves a "strong understanding" of how evolutionary processes (e.g. dispersal, migration, genetic drift, natural selection, mating systems, habitat structure, and geographic isolation) function in the species of interest (Winston 1999:325). While naming subspecies can aid conservation by emphasizing "evolutionarily valuable genetic resources within species that might vanish even if other populations of the species survive" (Winston 1999:324), Mayr (1982:107) cautions against over splitting, which can "obscure" the presence of intraspecific variation.

The similarity of the Alaska Peninsula haplogroup to *L. timidus* haplotypes and the presence of northern hares on Little Diomede Island in the Bering Strait suggest there may have been limited gene flow with *L. timidus* since the geographic "separation" of the 2 taxa. Overall, arctic sea ice extent has been decreasing with climate change, but ice in the Bering Sea has increased in recent years (National Snow and Ice Data Center 2012), and it is unclear how large of a role these changes will play for potential gene flow between Alaskan and Mountain Hares. The confounding factor of tundra habitat loss at high latitudes (Murphy et al. 2010) may also contribute to changes in northern hare phylogeography in the near future. In Alaska, the western tundra biome is predicted to decrease by 54%, "all but disappearing" in its current location by

the end of the 21st century (Murphy et al. 2010:4). Additionally, almost all of western Alaska north of the Alaska Peninsula is predicted to undergo at least 1 biome shift (Murphy et al. 2010).

Our research reveals more mitochondrial diversity than previously reported in *L. othus*, a new biogeographic pattern and closer mtDNA association with *L. timidus*, and possible recent or ongoing gene flow across the Bering Strait. It also highlights the importance of maximizing sample sizes and sampling widely across a taxon's geographic distribution. Previous molecular research on *L. othus* (Waltari and Cook 2005; Melo-Ferreira et al. 2012) did not detect the Alaska Peninsula haplogroup because the single Alaska Peninsula sequence (UAM 42143) included in both studies (GenBank accession AY422251) represented a haplotype shared with the rest of the Alaskan Hare's distribution. Consequently, these studies recovered *L. othus* as monophyletic within the northern hare complex (Waltari and Cook 2005; Melo-Ferreira et al. 2012). Quantifying the changes in arctic phylogeography during the Pleistocene can better inform us about the evolutionary processes that have shaped the biogeographic patterns we observe in the Holocene and how those patterns are likely to be affected by an increasingly changeable Arctic.

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## TABLES

Table 2.1.—Analysis of Molecular Variance (AMOVA) for *Lepus othus* populations grouped by subspecies, groups with highest $\Phi_{ST}$ (varianLce among subpopulations relative to the total variance), and groups with the highest $\Phi_{CT}$ (variance among groups relative to total variance).

| *Groups* | *Source of variation* | *% variation* | *Φ* | *p-value of Φ* |
|---|---|---|---|---|
| *L. o. othus* vs. *L. o. poadromus* | Between groups | 53.82 | $\Phi_{SC}$=0.53 | 0.00 |
| | Among populations within groups | 24.54 | $\Phi_{ST}$=0.78 | 0.00 |
| | Within populations | 21.64 | $\Phi_{CT}$=0.54 | 0.13 |
| Alaska Peninsula vs. rest of Alaska | Between groups | 58.82 | $\Phi_{SC}$=0.55 | 0.00 |
| Alaska Peninsula vs. rest of Alaska | Among populations within groups | 22.79 | $\Phi_{ST}$=0.82 | 0.00 |
| Alaska Peninsula vs. Bristol Bay | Within populations | 18.38 | $\Phi_{CT}$=0.59 | 0.16 |
| vs. Kuskokowin+Yukon Deltas vs. Little Diomede vs. Seward Peninsula | Among groups | 69.52 | $\Phi_{SC}$=-0.01 | 0.45 |
| Alaska Peninsula vs. Bristol Bay | Among populations within groups | -0.23 | $\Phi_{ST}$=0.69 | 0.00 |
| vs. Kuskokowin+Yukon Deltas vs. Little Diomede vs. Seward Peninsula | Within populations | 30.71 | $\Phi_{CT}$=0.70 | 0.06 |

Table 2.2.—Population comparisons of *Lepus othus*. Pairwise $\Phi_{ST}$ values are above the diagonal and corrected average pairwise differences are below the diagonal.

| | Alaska Peninsula | Bristol Bay | Kuskokwim Delta | Yukon Delta | Seward Peninsula | Little Diomede |
|---|---|---|---|---|---|---|
| Alaska Peninsula | | 0.16 | 0.70* | 0.69* | 0.86* | 0.52* |
| Bristol Bay | 1.89 | | 0.28 | 0.25* | 0.71* | 0.21 |
| Kuskokwim Delta | 14.76* | 1.96 | | -0.02 | 0.57* | 0.75* |
| Yukon Delta | 15.21* | 2.27 | -0.09 | | 0.64* | 0.76* |
| Seward Peninsula | 15.59* | 4.68* | 2.63* | 3.39* | | 0.89* |
| Little Diomede | 11.00* | 6.74 | 12.01* | 12.57* | 13.35* | |

* $P < 0.05$

Table 2.3.—Molecular diversity statistics: sample size ($n$), number of segregating sites ($S$), number of haplotypes ($h$), haplotype diversity ($H_d$), per-site nuclear diversity ($\pi$), and mean number of pair-wise differences ($d_x$). Changes in historical population size measured by Tajima's $D$ and Fu's $Fs$.

| *Population Name* | *n* | *S* | *h* | *$H_d$* | *$\pi$* | *$d_x$* | *Tajima's D* | *Fu's Fs* |
|---|---|---|---|---|---|---|---|---|
| Alaska Peninsula | 10 | 27 | 5 | 0.756 +/- 0.130 | 0.014 +/- 0.008 | 9.089 +/- 4.573 | -0.228 | 3.325 |
| Bristol Bay | 5 | 30 | 5 | 1.000 +/- 0.127 | 0.026 +/- 0.017 | 16.80 +/- 9.048 | 1.246 | 0.315 |
| Y-K Delta | 22 | 14 | 6 | 0.688 +/- 0.098 | 0.006 +/- 0.004 | 3.909 +/- 2.038 | 0.064 | 2.018 |
| Seward Peninsula | 80 | 15 | 14 | 0.750 +/- 0.039 | 0.003 +/- 0.002 | 1.709 +/- 1.009 | -1.243 | -5.418* |
| Little Diomede | 2 | 3 | 2 | 1.000 +/- 0.500 | 0.004 +/- 0.005 | 3.000 +/- 2.449 | - | - |
| Total *L. othus* population | 119 | 47 | 27 | 0.858 +/- 0.023 | 0.010 +/- 0.001 | 6.961 +/- 3.210 | -0.644 | -2.766 |

* $P < 0.02$

Table 2.4.—Mismatch distributions for populations and *Lepus othus* as a whole. The sample size from Little Diomede was too small to analyze. $\tau$ is an estimate of the mode of the distribution relates to the time since the population expansion with the equation $\tau=2\mu t$ where $\mu$ is the mutation time of *Lepus* mtDNA control region (0.00012). $\tau$ and the time since population expansion are only determined if the population's mismatch distribution does not significantly differ from the sudden-expansion model.

| *Population* | *τ and 95% CI* | *Years since expansion* |
|---|---|---|
| Alaska Peninsula | 0 (0.000, 4.119) | 0.00 |
| Bristol Bay* | 24.600 (15.330, 109.599) | - |
| Y-K Delta* | 8.324 (1.316, 61.324) | - |
| Seward Peninsula | 2.145 (0.666, 4.791) | 17,944.38 |
| All *L. othus* | 28.736 (0.000, 113.736) | 240,396.2 |

* $P < 0.05$

Table 2.5.—Names of craniodental measurements, definitions, ICC scores of repeatability, within measurement variance, *P*-values for Wilcoxon rank sum tests, slopes, and R2 values of the linear fit by latitude. Only measurements that were determined to be repeatable and reproducible are included.

| Measurement | Description according to Anderson (1974) | *ICC* score with 95% CI | Variance | Wilcoxon test *P*-value | *Slope* | $R^2$ |
|---|---|---|---|---|---|---|
| Greatest Length I | Anteriormost face of upper incisors to posterior border of interparietal. | 0.9757 (0.9283, 0.9970) | 0.0665 | 0.1875 | 0.00213 | 0.305765 |
| Basilar Length | Posterior edge of alveolus of $I^2$ to inferior border of the foramen magnum. | 0.9827 (0.9482, 0.9979) | 0.0362 | 0.9184 | 0.0024567 | 0.209233 |
| Length of Nasals | Greatest diagonal length of the longest nasal | 0.9836 (0.9509, 0.9980) | 0.0313 | 0.2027 | 0.0016777 | 0.051132 |
| Width of Nasals | Greatest breadth of nasals near posterior border. | 0.9953 (0.9855, 0.9994) | 0.0079 | 0.6818 | 0.0013005 | 0.030145 |
| Maxillary Tooth Row | Length of maxillary tooth row at the alveolar border. | 0.9113 (0.7654, 0.9886) | 0.0461 | 0.65 | 0.0017136 | 0.131445 |
| Zygoma Length | Maximum length of the zygomatic arch. | 0.9899 (0.9694, 0.9988) | 0.0273 | 0.3266 | 0.00218 | 0.138569 |
| Greatest Length II | Anteriormost face of upper incisors to the posterior edge of external occipital protuberance. | 0.9912 (0.9731, 0.9989) | 0.0269 | 0.4553 | 0.0027526 | 0.375581 |
| Condylobasal Length | Posterior edge of occipital condyles to the anteriormost face of incisors. | 0.9708 (0.9146, 0.9964) | 0.1119 | 0.7143 | 0.0027784 | 0.377083 |
| Length of Palatal Bridge | Greatest length of bony palate. | 0.9239 (0.7944, 0.9903) | 0.0152 | 0.9649 | 0.0037112 | 0.100453 |
| Greatest Length of Mandible | Posterior edge of mandible to anteriormost face of incisors. | 0.9649 (0.8984, 0.9957) | 0.1041 | 0.9883 | 0.0025944 | 0.31399 |
| Greatest Depth of Mandible | Top of articular to bottom of angle. | 0.9653 (0.8995, 0.9958) | 0.1404 | 0.5484 | 0.0020733 | 0.154079 |

Table 2.6.—Eigenvalues and percent of variance accounted for by each principal component (PC).

| *PC* | *Eigenvalue* | *% variance* | *Cumulative %* |
|---|---|---|---|
| 1 | 0.0022 | 48.023 | 48.023 |
| 2 | 0.0010 | 22.296 | 70.319 |
| 3 | 0.0004 | 8.946 | 79.266 |
| 4 | 0.0003 | 7.405 | 86.670 |
| 5 | 0.0002 | 4.798 | 91.468 |
| 6 | 0.0001 | 3.000 | 94.468 |
| 7 | 0.0001 | 2.405 | 96.873 |
| 8 | 0.0001 | 1.632 | 98.506 |
| 9 | 0.0000 | 0.791 | 99.297 |
| 10 | 0.0000 | 0.467 | 99.764 |
| 11 | 0.0000 | 0.236 | 100.000 |

Table 2.7.—Loading matrix of the first 4 principal component (PC) coefficients.

| **Variable** | **PC 1** | **PC 2** | **PC 3** | **PC 4** |
|---|---|---|---|---|
| Greatest Length I | 0.79923 | 0.37123 | 0.22210 | 0.00556 |
| Basilar Length | 0.66795 | 0.31474 | 0.40336 | 0.06360 |
| Length of Nasals | 0.66442 | 0.25622 | -0.25943 | -0.64794 |
| Width of Nasals | 0.54496 | 0.46778 | -0.60501 | 0.31926 |
| Maxillary Tooth Row | 0.58495 | 0.33770 | 0.11884 | 0.22802 |
| Zygoma Length | 0.62253 | 0.18323 | 0.31993 | -0.08142 |
| Greatest Length II | 0.82686 | 0.35931 | 0.27366 | 0.02917 |
| Condylobasal Length | 0.80450 | 0.37402 | 0.26988 | 0.04576 |
| Length of Palatal Bridge | 0.73726 | -0.67152 | -0.05518 | 0.04835 |
| Greatest Length of Mandible | 0.77046 | 0.36417 | 0.24214 | 0.09164 |
| Greatest Depth of Mandible | 0.66349 | 0.34302 | 0.13498 | 0.20657 |

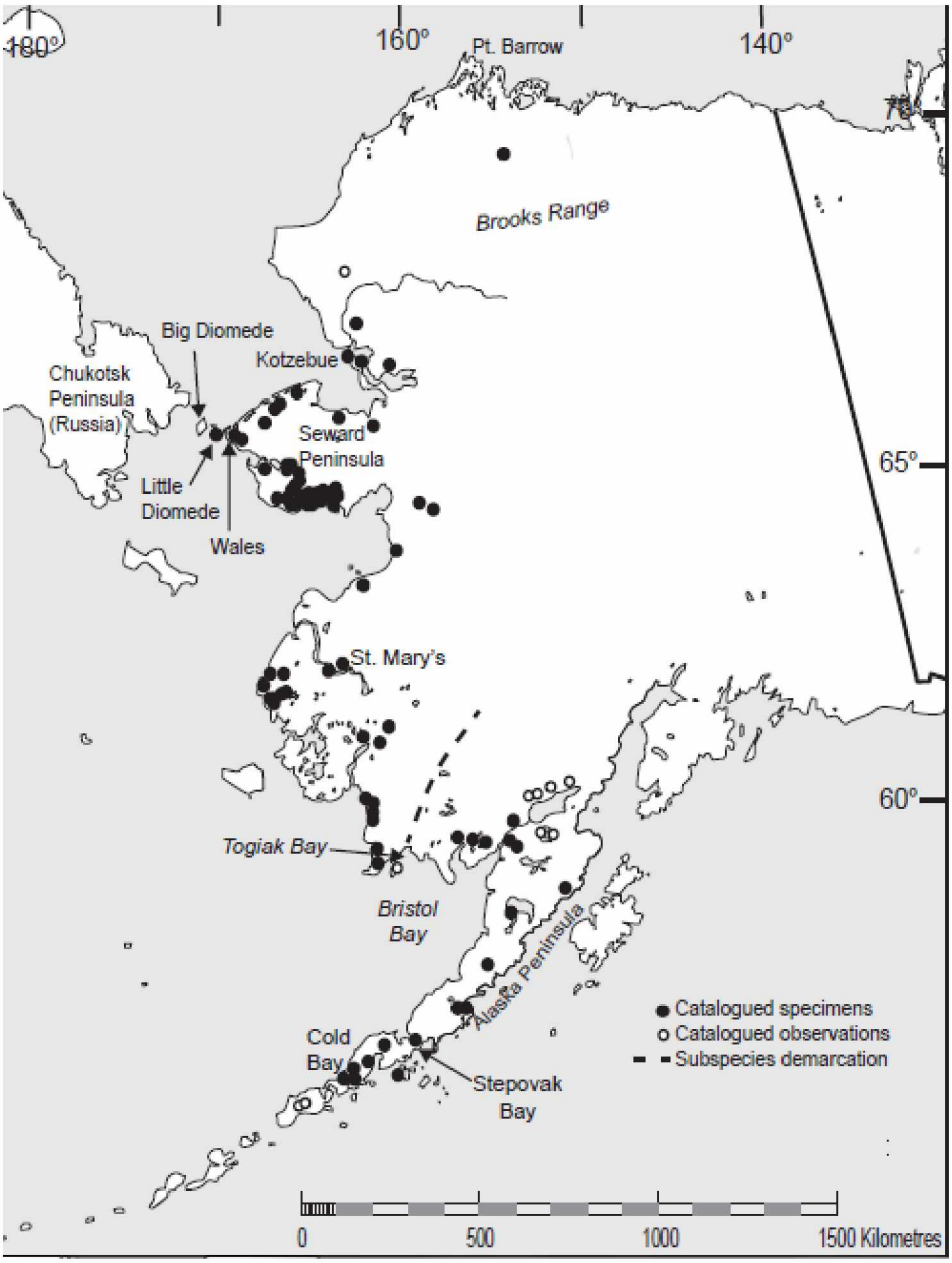


Figure 2.1.—*Lepus othus* distribution.

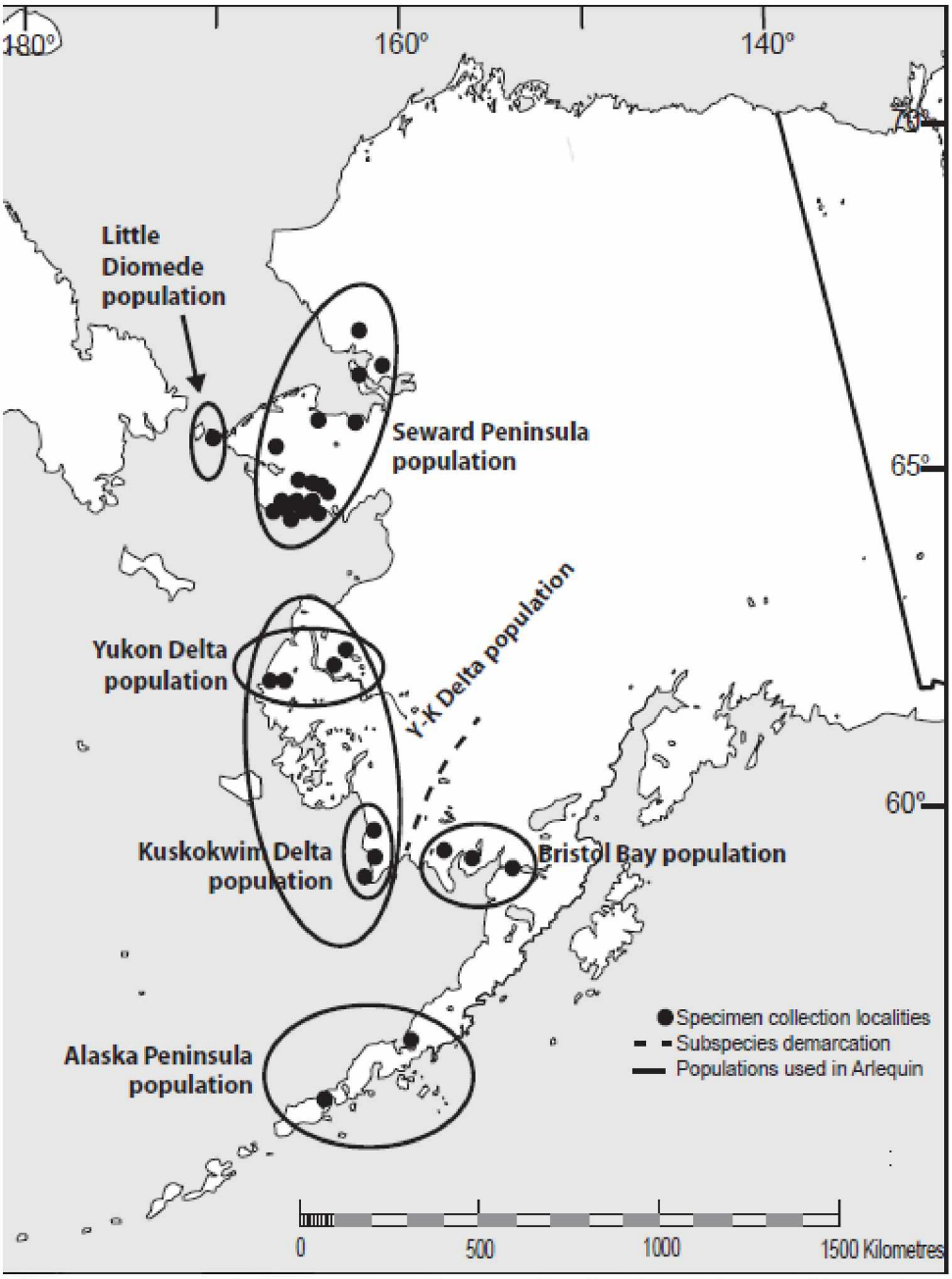

Figure 2.2.—Populations and specimen collection localities used in molecular analyses.

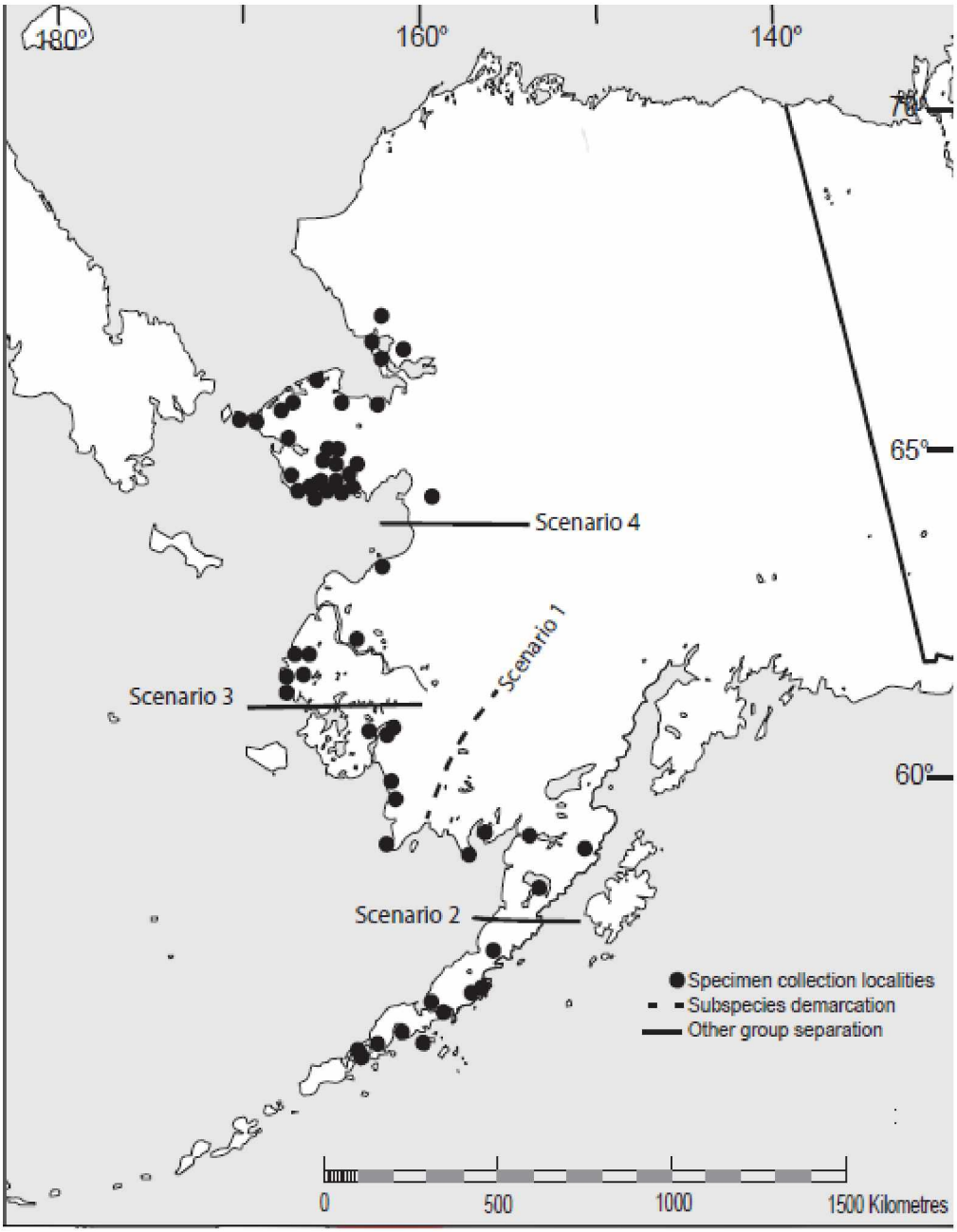

Figure 2.3.—Collection localities of specimens used in morphometric analyses, subspecies demarcation, and north/south groupings used in discriminant function analysis.

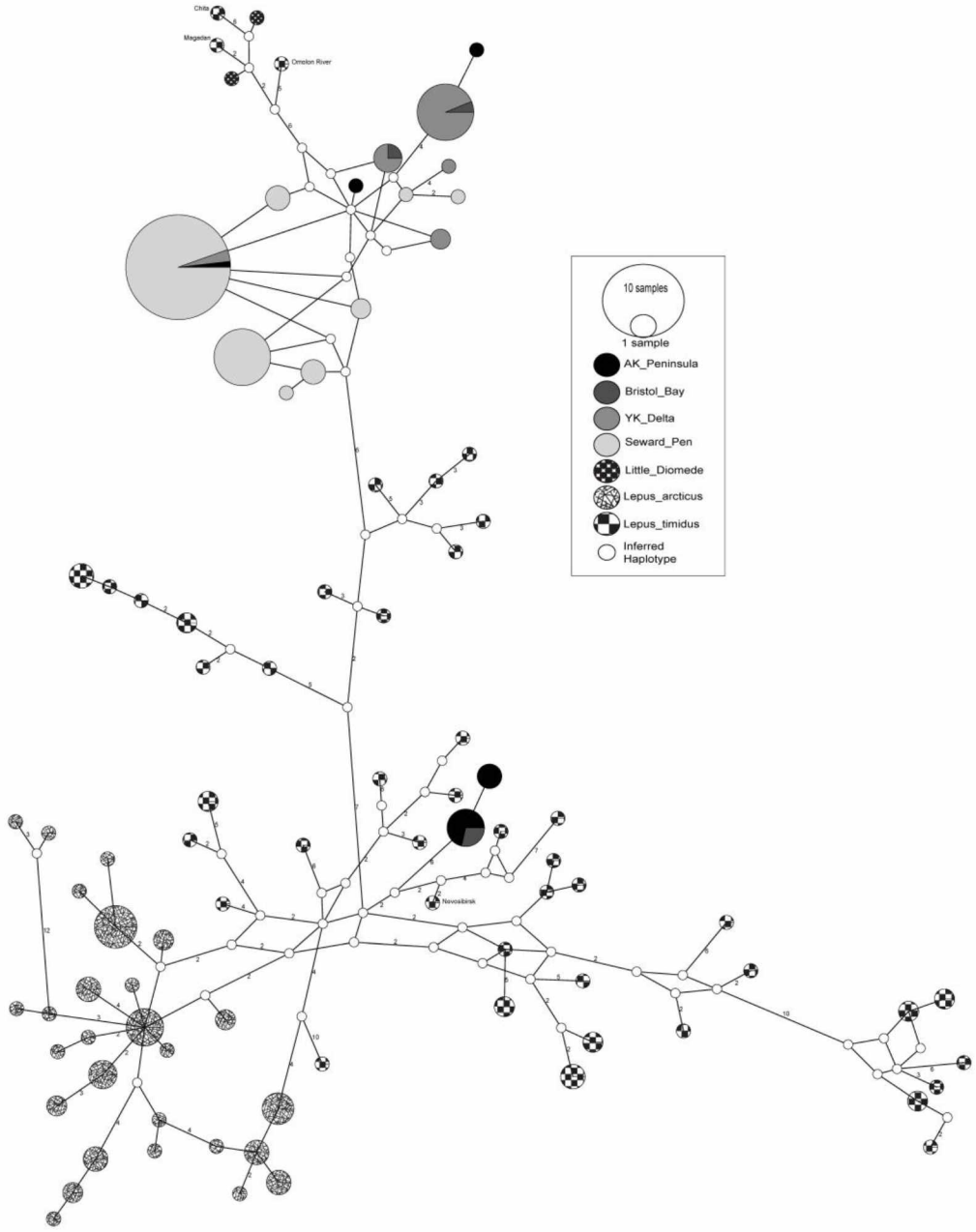


Figure 2.4a.—Median-joining network. Each circle represents a haplotype, and each Alaskan Hare population and northern hare species has a different color or grayscale pattern. Inferred haplotypes are open circles. The larger the circle, the more specimens of that haplotype.

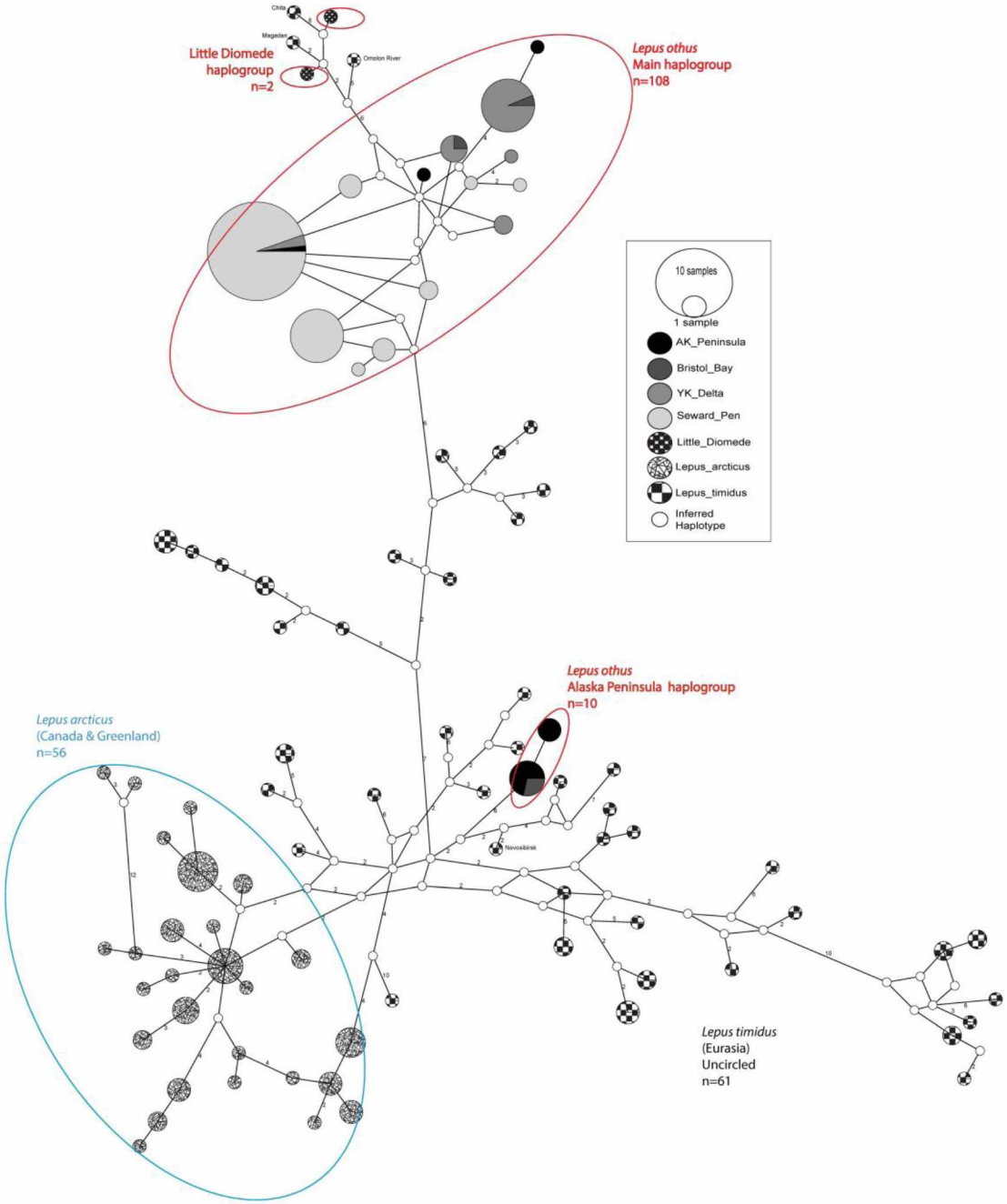


Figure 2.4b.—Median-joining network with haplogroups and species indicated. World-wide occurrence follows the species name in parentheses and n = the size of each haplogroup.

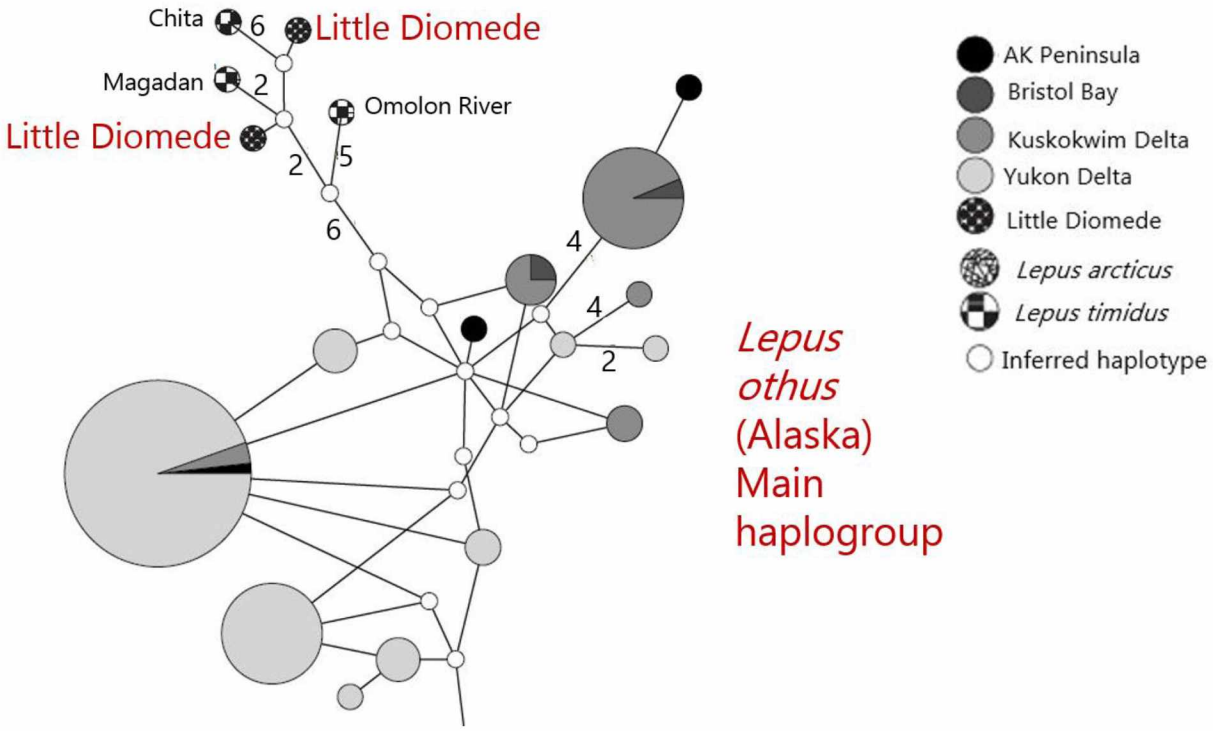


Figure 2.4c.—Close-up of the median-joining network. This includes the main *Lepus othus* haplogroup, the Little Diomede specimens, and *L. timidus* specimens with similar mtDNA control region sequences. Each circle represents a haplotype, and each Alaskan Hare population and northern hare species has a different color or grayscale pattern. Inferred haplotypes are open circles. The larger the circle, the more specimens of that haplotype. The numbers adjacent to the branches are the number of nucleotides that differ between each haplotype. If there is not a number next to a branch, that branch represents a single nucleotide difference between haplotypes.

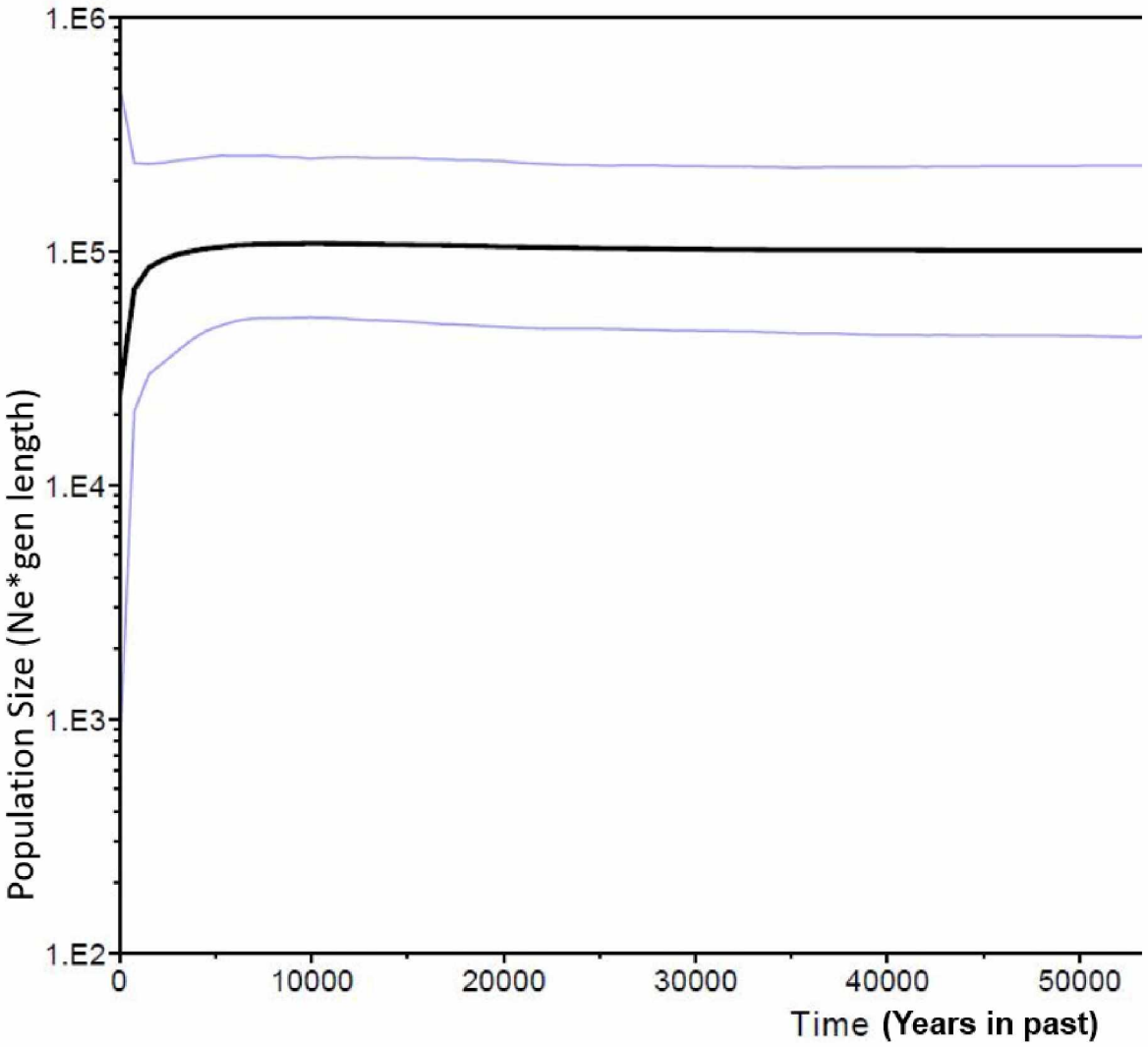


Figure 2.5.—Bayesian skyline plot of *Lepus othus*: population size (in units of effective population size times the generation length of 2 years) over time (in years in the past). The center bold line represents median population size, and the 95% highest posterior density intervals extend to the outer lines. The BSP analysis indicates a possible slight and recent demographic decline, but overall shows little demographic change over time.

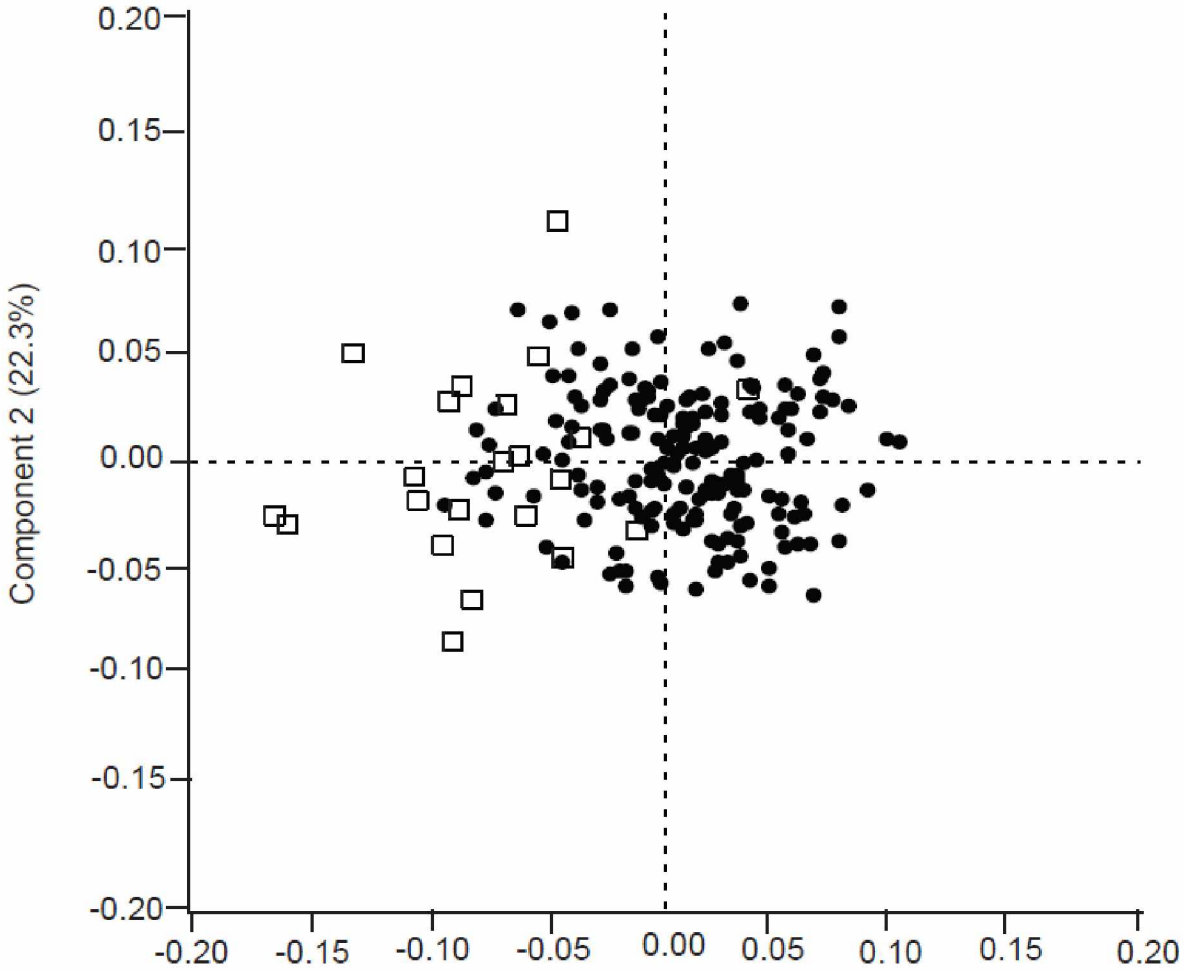


Figure 2.6.—Plot from principal components analysis. Principal component 1 accounts for 48% of the variance and principal component 2 accounts for 22.3% of the variance. There is noticeable overlap between the 2 subspecies.

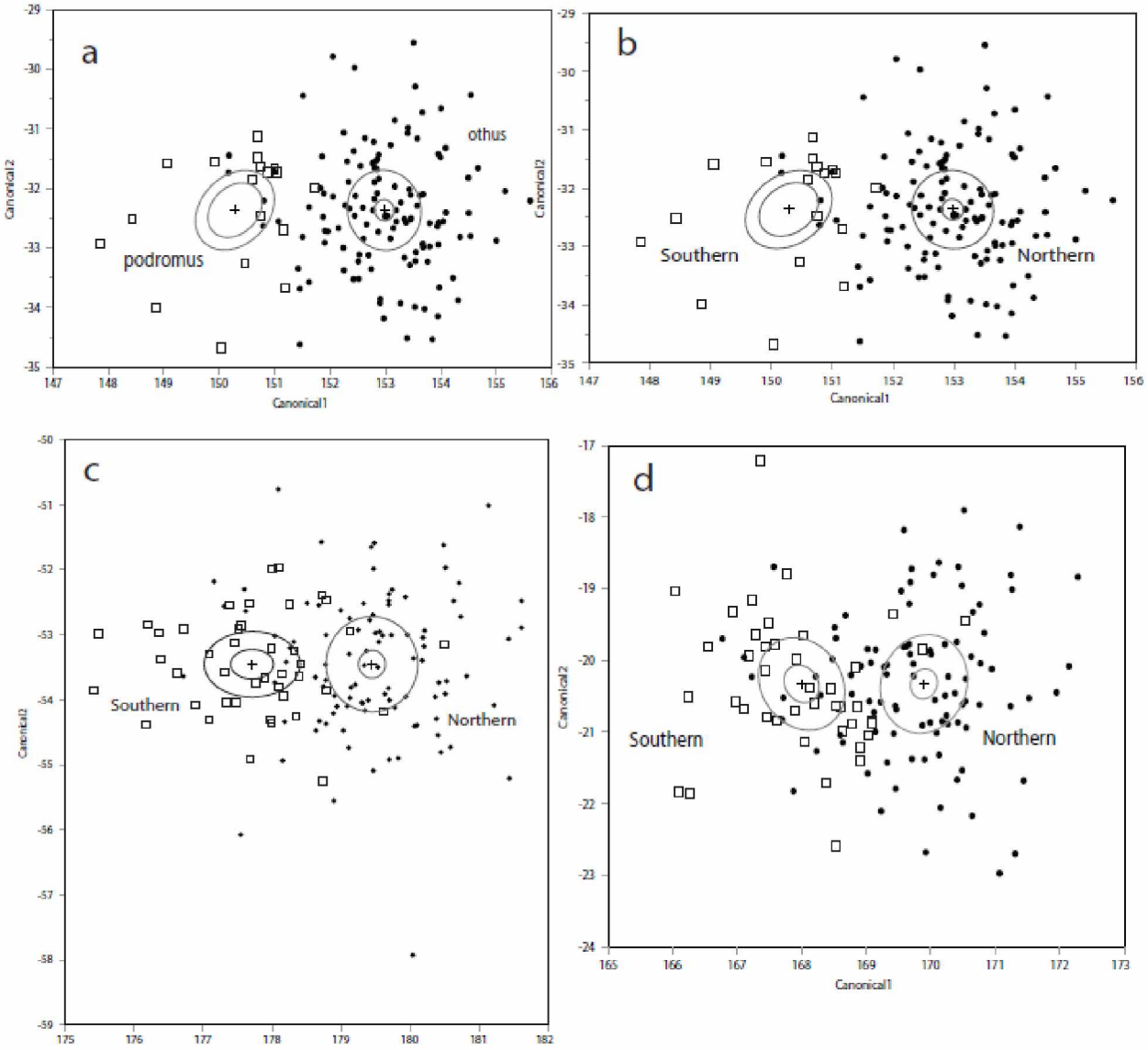


Figure 2.7.—Series of discriminant function analysis canonical plots with specimens grouped by (a) subspecies, (b) 57°N, (c) 61°N, and (d) 64°N. Open boxes represent specimens of the southern group, filled dots represent specimens of the northern group, the plus signs represent each multivariate mean, the inner ellipse around the plus sign denotes the 95% confidence level for the mean, and the outer ellipse denotes the 50% confidence level.

# CONCLUSIONS

We have updated the Alaskan Hare's current northernmost range limit to approximately 50 km northeast of Kotzebue and located the "lost" McIlhenny specimen, the only *L. othus* specimen with a collection locality from the North Slope. Long-distance dispersal has been documented in northern hares (Angerbjörn & Flux 1995) and it is likely *L. othus* individuals have occasionally dispersed well outside of the species' current range. However, it is possible the hare was killed elsewhere and brought to the North Slope, perhaps via a series of trades. Since it remains the only known specimen to have been collected from the North Slope and its collection locality cannot be confirmed, we conclude there is insufficient evidence to include the North Slope in the current or recent distribution of *L. othus*.

Traditional Alaska Native trade routes and fairs were common until the 20th century (Burch 1988) and pelts (USNM E89915-0, USNM E89915-1) from the International Polar Expedition are evidence that hare skins were traded between Point Barrow and inland Eskimos (Murdoch 1885). However, these Snowshoe Hare pelts seem to have been misidentified Alaskan Hares, at the time given the common name of polar hares. The name "Nunatamium" is also written on the pelt skin tags. The Nunataaġmiut Iñupiat did live in the Colville Valley but were often confused with the Nuataaġmiut Iñupiaq of the inland Noatak River or the Napaaqtuġmiut Iñupiaq of the Noatak Basin (Burch 1998). All 3 of these groups traded with one another and with Point Barrow residents, but only the Iñupiaq lived close to what is now the northern extent of the Alaskan hare's distribution (Burch 1998). It is possible that dubious field identifications and confusion about the movements of the various Alaskan Native groups in northern Alaska may have contributed to the anecdotal accounts of *L. othus* occurring on the North Slope.

Even today not all sources accurately differentiate between *L. othus* and *L. americanus*. Subsistence harvest records do not always distinguish between hares species (Alaska Department

of Fish & Game 2014b), and *L. othus* and *L. americanus* may be misidentified in subsistence reporting. Therefore, it is difficult to determine if local harvests have changed over time and if they reflect the decline of *L. othus* suggested by Klein (1995). Additionally, any of 4 food resource categories recognized by ADFG—arctic hare, hare, jackrabbit, and unknown hare—could include *L. othus*. State hunting and trapping regulations erroneously list "snowshoe hare" and "arctic hare" as the 2 species of hare in Alaska (Alaska Department of Fish & Game 2014b:133), which reflects both the widespread taxonomic confusion surrounding northern hares and the challenges with common names. Updated distributional data substantially aids wildlife management decisions as industrial development and climate change continue to effect Arctic environments.

Notable additions to the Alaskan Hare's known distribution include the recent specimens collected from Little Diomede Island (UAM 120797 and UAM 122839). Although hares were uncommon on Little Diomede in recent years, they were frequently observed several human generations ago (Haeker 2014). Today's elders remember their own elders talking about hares and foxes crossing back and forth between Little and nearby Big Diomede Islands. One hare was purportedly hunted in the 1970s, but by then such an event was a rare occurrence (Haeker 2014). Thus, presumably infrequent but recurrent gene flow between the two species may be facilitated by island stepping stones and the decreasing extent of sea ice in the Bering Strait may present a barrier to a gene flow pathway between Eurasian and American northern hares.

*Lepus othus* is divided into 3 haplogroups that roughly correspond with geography: Little Diomede, Alaska Peninsula, and the rest of western Alaska. The Alaska Peninsula and Little Diomede haplogroups are more similar to some *L. timidus* haplotypes than to the other *L. othus* haplogroups, which may be a result of immigration or introgression from western Beringia, from

mitochondrial sweep of dispersers as land ice retreated, from a mitochondrial sweep that has occurred during the Holocene, or from incomplete lineage sorting. The Bristol Bay area had the highest levels of genetic diversity and appears to be a mixing zone between the Alaska Peninsula and the rest of mainland western Alaska. However, because the species limits between *L. othus*, *L. timidus*, and *L. arcticus* remain murky, we cannot accurately determine *L. othus* subspecies.

Future research with northern hares will require genomic sequencing to resolve species and subspecies limits. The validity of *L. othus* and its subspecies can affect potential conservation efforts, especially if tundra continues to shrink with rising global temperatures and gene flow is restricted by habitat fragmentation. Further resolution of northern hare systematics and population size estimates are necessary before we can determine the Alaskan Hare's baseline global distribution or conservation status, let alone predict its future reactions to the changing Arctic.

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